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A STUDY OF COMBINING ABILITY OF ALFALFA IN RELATION TO CERTAIN METHODS OF SELECTION¹

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INTRODUCTION

In Saskatchewan alfalfa is rapidly becoming an important crop particularly in the central and northern portions of the province where moisture conditions are favorable. According to census reports (14) the alfalfa acreage in Saskatchewan in 1931 was 6,860. By 1941 this had increased to 113,183. Acreages in the other two prairie provinces of Alberta and Manitoba, and throughout Canada as a whole, have followed this same general trend. Much of the expanded acreage, particularly in Saskatchewan, resulted from the development of seed production in northern districts such as the White Fox area about 200 miles north-east of Saskatoon. It is estimated that about 50 per cent of the total alfalfa acreage in the province is devoted to seed production.

Two strains of Grimm, Sask. No. 451 and Sask. No. 666, account for over 90 per cent of the seed produced in Saskatchewan. Both strains were selected at the University of Saskatchewan. Sask. No. 451 is a mass selection of hardy plants and Sask. No. 666 was increased from a single plant selected for high seed yield (30). Ladak is well adapted and so is Cossack but these varieties are not generally grown because of the market demand for Grimm seed.

As long as production was confined to a few growers with small fields, seed yields were generally high and 300 to 400 pounds per acre not uncommon. During the past five to ten years, however, 200 to 300 pounds per acre is considered a good yield. Soil fertility, harmful and beneficial insects, plant diseases and the climatic factors of temperature and moisture all have played their part in this decrease in yield. Variation between individual plants and strains for almost all agronomic characters is very great and improvement of the crop should be possible by proper breeding and selection methods. However, the best methods for the improvement of a cross-fertilized perennial crop such as alfalfa are not well established. The purpose of this study was to test certain methods of selecting parental material and to determine suitable means for evaluating their use in a breeding program. While seed yield was the main criterion used, it is believed that any principles established can be applied to selection and breeding for other characters.

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LITERATURE REVIEW

Several authors have published reports on the genetics of alfalfa. Waldron (55) Lepper and Odland (39) and Korohoda (36) studied the inheritance of difference in flower color in the cross between *Medicago falcata* and *M. sativa* and each concluded that 2 or more factor pairs were involved. Armstrong and Gibson (1) reported segregation for flower color, pod hairiness, growth habit, stoloniferous habit and leaf size and shape in the cross between *M. media* and *M. glutinosa*. They assumed 3 factor pairs (one of which was homozygous) to explain the purple flower color of the *M. media* parent. To explain the cream flower color of the *M. glutinosa* parent they assumed an additional factor pair allelomorphous to the homozygous pair conditioning purple flower color in *M. media*. Hairiness of the pod appeared to be generally controlled by one factor pair but exceptional ratios were explained on the basis of a pair of inhibiting factors. At least two factor pairs appeared to condition growth habit and the stoloniferous habit was conditioned by at least three factor pairs. Leaf size and shape appeared to be dependent on multiple factors. MacVicar (40) reported that the assumption of at least three factor pairs was required to explain the inheritance of differences in the intensity of black seed coat color but that lack of seed coat color was simply inherited. Brink, Jones and Albrecht (7) concluded that the inheritance of resistance to bacterial wilt in alfalfa probably was complex genetically. Tysdal *et al.* (50) found that Korohoda's data on leaf shape gave a very close fit to calculated autotetraploid ratios. They concluded that common alfalfa probably is a tetraploid and that some evidence indicated it to be autotetraploid. Hence they suggest that tetrasomic ratios are the most likely to provide satisfactory fits to genetical data in common alfalfa.

Fryer (18) has reported an extensive study of the cytology of the genus *Medicago*. He found that the somatic chromosome number of the several species studied was either 14, 16 or 32. In *M. falcata* both 16- and 32-chromosome forms occurred. He suggested that the 32-chromosome species arose from 16-chromosome species by tetraploidy. Ledingham (37) also found 16- and 32-chromosome forms of *M. falcata* and he crossed the 16-chromosome form with *M. sativa* ($N=32$). When *M. sativa* was the ♀ parent only 2 hybrid plants were obtained although a large number of flowers were pollinated. Both of these hybrid plants proved to be triploids. When *M. falcata* was the ♀ parent several hybrids were obtained which later proved to be tetraploids. These tetraploids were relatively self-sterile but were cross-fertile when crossed back to *M. sativa*. In these backcrosses to *M. sativa* pairing was good but not always complete, univalents frequently being present. The conclusion reached was that the *falcata* and *sativa* chromosomes are homologous and interpair freely.

The amount of cross-fertilization occurring under natural conditions has been reported from widely separated points. Burkart (9) in Argentina, found an average of 84.5 per cent with a range from 67 to 98 per cent. Tysdal *et al.* (50) in the United States, got an average of 89 per cent with a range of 83 to 97 per cent, and Knowles (34) in western Canada, reported an average of 94 per cent cross-fertilization and a range from 91 to 100 per cent. However, although alfalfa is cross-pollinated normally, it may show considerable self-fertility when artificially self-pollinated. Bolton and

Fryer (5) found that the average number of pods set based on the number of flowers tripped was 3.8 per cent for a group of 9 sterile plants as compared to 54.7 per cent for a group of 11 fertile plants. Similarly Knowles (34) reported an average of 1.65 seeds per flower tripped when a group of 10 high seed-setting selections were self-pollinated as compared to an average of 0.56 seeds per flower tripped on self-pollinating a group of 10 random plants of the Grimm variety. Tysdal *et al.* (50) emphasizes the variation in the self-fertility of different alfalfa plants and suggests a practical use for self-sterility in a breeding program. Cooper and Brink (11) found that the pollen tubes of foreign pollen grew faster and were more likely to effect fertilization than the pollen tubes of self pollen. This provides a reasonable explanation of the high percentage of cross-pollination reported even though a considerable degree of self-fertility exists.

It has been demonstrated that self-fertilization results in a progeny that is likely to show a considerable decrease in forage yield and an even greater decrease in seed yield. Kirk (32) reported the average yield of fourth generation selfed lines to be 54 per cent for forage and 22 per cent for seed when expressed as a percentage of a standard variety. Comparable results presented by Tysdal *et al.* (50) are 51 per cent forage and 36 per cent seed for fourth generation and 28 per cent forage and 8 per cent seed for eighth generation selfed lines, also expressed as a percentage of standard varieties. Both Kirk and Tysdal *et al.* reported on hybrid progenies from inbred parents. Kirk noted one such progeny in particular which provided an excellent example of hybrid vigor expressed in seed and forage yield. Tysdal *et al.* found that certain hybrids showed a decided amount of hybrid vigor while others did not, thus indicating differences in combining ability. They presented data for four inbred lines with a mean forage yield of 55 per cent of three standard check varieties. Two F_1 single crosses from these inbreds averaged 96 per cent and the F_2 progenies only 41 per cent. However, another experiment involving an inbred line and a clone, not inbred, showed no appreciable reduction in the yield of the F_2 .

"Tripping" in alfalfa is a term applied to the process whereby the staminal column is released from the tissues attaching it to the keel and wing petals and strikes against the banner petal. The mechanism of this process in normal and self-tripping alfalfa flowers has been described by Armstrong and White (2). They reported that tripping causes the rupture of a thin membrane covering the stigma and that unless this stigmatic membrane is ruptured it is an effective bar against pollen tube penetration. Brink and Cooper (6) do not agree that tripping is essential before seed-setting can take place and Carlson (10) has reported considerable seed-setting without tripping. Recently, however, Tysdal (48) reports that less than 5 per cent of the flowers set seed without tripping. Also he has suggested that the difficulty in distinguishing between "tripped wrapped" flowers and untripped but wilted flowers may explain the relatively large amount of seed-setting without tripping which Brink and Cooper reported. All writers agree, at least, that tripping is a very important factor in seed-setting.

The factors that affect tripping have received intensive study. Gray (22) found that wind tripped alfalfa blossoms only under the most unusual circumstances. Ufer (52) reported that wind increased the favorable effect

of heat but attributed it to the drying action. Knowles (34) found no correlation between tripping and wind velocities and Tysdal (48) considered wind unimportant. Tysdal found, however that 8.3 per cent of the flowers were tripped by rain and Knowles also stated that a rain shower increased tripping. Both authors, though, considered rain unimportant as little seed was set as a result of this tripping since it appears that crossing does not occur during rain. Ufer (52) concluded that heat caused tripping and that the process was accelerated by a drying action, although drying of itself did not cause tripping. Dwyer and Allman (15) found that alfalfa flowers tripped instantaneously at temperatures ranging between 100° F. and 108° F. and that the effect was similar whether dry heat, steam or hot water was used. Several authors (17, 43, 52, 15) all found that tripping could be readily initiated by exposing the flowers to direct heat supplied by a lighted match, lighted cigarette, or burning glass. Knowles (34) found temperature the most important weather factor influencing the tripping of alfalfa flowers.

On the effect of insects on tripping there is general agreement (43, 17, 51, 34, 46, 41) that leaf cutter bees and bumble bees are effective tripping agents. Tysdal (46) also found that bees belonging to the genus *Nomia* were important. There is no similar agreement as to the value of honey bees. Gray (22) Piper *et al.* (43) Englebert (17) Knowles (34) and Peck and Bolton (41) reported that honey bees were unimportant. On the other hand, Dwyer and Allman (16) and Lejeune and Olson (38) found considerable numbers of tripped flowers due to honey bees. Recent articles by Hare and Vansell (24) and Vansell and Todd (54) showed that honey bees may be very effective trippers when they are collecting pollen and that pollen collecting was influenced by the relative abundance of competing flowers and the abundance and attractiveness of the alfalfa.

Factors inherent in the plant itself may also have an effect on tripping. Armstrong and White (2) have described self-tripping alfalfa plants and have compared them to normal types. The performance of these self-tripping plants and their progenies in field plot experiments have been reported by Stevenson and Bolton (45). However, it appears that the self-tripping character is not common and adds little to the amount of tripping observed in ordinary fields of alfalfa. The easy-tripping plants mentioned by Tysdal (48) very probably are a quantitative expression of the self-tripping described by Armstrong and White.

It is frequently suggested, particularly by commercial seed growers, that mechanical trippers could be developed. Silversides and Olsen (44) tested several methods of mechanical tripping. They found that while these trippers sharply increased the amount of tripping they usually resulted in a decreased seed yield. The decrease in seed yield was attributed to rather severe damage to the plants by the tripping devices.

Commercial fertilizers have been shown (4) to increase markedly the yield of both seed and forage of the alfalfa crop. In practically all experiments sulphur bearing fertilizers were the only ones which affected yield.

Of the several diseases affecting alfalfa, bacterial wilt is probably the most important. The disease was described originally by Jones and McCullough (27) in 1925 and the causal agent identified as *Aplanobacter insidiosum*. Bacterial wilt is widely distributed in North America particularly in irrigated soils and areas with good moisture conditions. The variety Turkestan is highly resistant and varying degrees of resistance are found in the varieties Ladak, Viking, Cossack and Hardistan. Two new varieties, Ranger and Buffalo, have been developed specifically for resistance to bacterial wilt and breeding is being continued at several institutions in the United States and Canada. Crown rot, described by Broadfoot and Cormack (8), and caused by an unnamed Basidiomycete, is a serious disease of alfalfa in western Canada. *Medicago falcata* shows some resistance (12) and breeding for resistance to the disease is now under way. Blackstem, caused by *Ascochyta imperfecta* is very prevalent in alfalfa fields. The actual effect of this disease on seed and forage yield has not been assessed. However, it is widely distributed (42, 13) and undoubtedly is responsible for considerable damage. Definite indications of variety and plant resistance have been noted (42). A species of rust, *Uromyces striatus* has been reported on alfalfa (35) and marked differences in resistance among varieties and species of *Medicago* was noted.

Methods of breeding for the improvement of alfalfa have been discussed by several writers. Kirk (29) stated that "selection within self-fertilized lines appears to provide a promising mode of attack for the breeding of improved varieties of alfalfa". In a later article (31), however, he concluded that the results from selfed line breeding had not been impressive and that strain-building as described by Jenkin (26) was probably a better method. This latter method emphasizes the use of both selfed and crossed progenies to evaluate the parental plants that are finally chosen as a basis for a particular strain. The maternal-line selection method for improving alfalfa has been proposed by Fryer (19). The method is based upon four-year cycles following each other indefinitely. In the first year about 80 progenies, each containing about 50 plants, are grown side by side from seed. In the second year all plants are scored from the density of pods on each plant and decidedly poor plants and progenies are destroyed. The surviving plants are again scored for fertility in the third year and about 100 of the best are selected late in the fall. These selections represent from 30 to 40 different progenies. In the fourth year all plants are removed from the field except about 80 of the 100 selections made the previous fall. These 80 plants are allowed to set seed under open-pollination and this seed is used to establish a new cycle of 80 progenies. More recently Tysdal and his associates (47, 49, 50) have outlined a method of breeding alfalfa based upon the procedure used in the production of commercial hybrids in corn. The main points in their method are (1) selection of parental plants by a polycross progeny test (2) the use of self-sterility to ensure cross-fertilization (3) vegetative propagation of the parental plants to obtain sufficient quantities of single cross seed. Theoretically the suggested method is not unduly expensive and the preliminary results reported suggest that it may prove a valuable method in the improvement of alfalfa.

MATERIAL AND METHODS

The majority of the selections used in this study originated in Grimm strains Sask. 451 and Sask. 666. No data are available on the relative distribution of the two strains as they are considered of equal agronomic value. Since both are highly variable and widely grown they provided a ready source of material. A field of Ladak at the University of Saskatchewan was the source of the remaining selections. This variety is of different origin and has slightly higher forage yield and greater winter hardiness than Grimm.

The selections of Ladak and most of those of Grimm were high seed-setting plants taken from fields setting a poor crop of seed since it was believed that such plants possessed inherent and desirable factors for seed-setting. However, since it has been suggested (50) that fields setting a good crop of seed may be a better source for selections, a number of plants were chosen in Grimm fields from which a good crop of seed was harvested. Three general groups of selections were used in these studies. The white-flowered group, used for experiments on cross-pollination, consisted of eight plants with pure white flowers selected in fields grown from open-pollinated seed. Plants with any trace of color in the buds or flowers were discarded. Two of these plants were chosen previous to 1942 from poor seed-setting fields and six were selected in 1942, four being from poor and two from good seed-setting fields. A second, or inbred group, was composed of plants that had been inbred one or more generations and had been selected for self-tripping and self-fertility. The third, or open-pollinated group, consisted of plants grown from open-pollinated seed. They were selected for high seed-setting. Two plants in the white-flowered group were sufficiently high seed-setters to be included in this group also.

The inbred material available was made up of plants and lines selected by the staff of the Forage Crops Laboratory at Saskatoon during the period 1938-41. This material originated from 300 to 400 plants selected for high seed yield in poor seed-setting fields of the Grimm variety grown in the Saskatoon and White Fox areas. Subsequently these field selections were further selected for self-tripping by the method described by Armstrong and White (2). On plants grown in the greenhouse during the winter months, several racemes on each plant were tabulated for the number of flowers per raceme and a final record was made of the number of pods set. Thus, since the flowers were not manipulated in any way and tripping insects were not present, the percentage of pods set from a given number of flowers was taken as a measure of self-tripping. This method also selects for self-fertility since no account is taken of flowers that are self-tripping but do not set pods due to self-sterility. First generation progenies were grown from selfed seed from these selected plants and, from certain plants selected in 1938, second generation progenies were grown from selfed seed. All progenies were grown in the field and observed for seed and forage yield, self-tripping and self-fertility and general agronomic value. In the fall of 1942 when the present studies were begun 33 of these progenies were available.

The open-pollinated material was selected in the fall of 1942. A total of 127 plants was chosen for high seed yield as judged by the number of pods set. Plants bearing a heavy set of pods were particularly noticeable

in poor seed-setting fields. Thirty-eight selections were from Ladak and 89 from Grimm. The Ladak plants and 58 of the Grimm came from poor seed-setting fields at Saskatoon and 31 Grimm plants came from good seed-setting fields in the White Fox area.

Certain general methods were used to select and handle the material and to conduct and analyse experiments.

During the month of September the field selections were transplanted to the greenhouse where they were placed in 8" or 10" pots. No dormancy period was found necessary and the plants flowered readily and matured a good crop of seed in November and December. If cut back early in January a second crop of flowers and seed was obtained in March and April. In May the plants were transferred to field plots where a third crop of flowers and seed developed. Greenhouse temperatures ranged from 50° to 60° F. at night and 70° to 80° F. during the day. From sunset to sunrise the plants were illuminated with one 500 watt Mazda lamp with reflector for every 5-foot square of pots. Good drainage was provided and the pots were soaked with water about every three days or often enough to keep the soil moist. Two or three applications, each of about $\frac{1}{2}$ teaspoonful of 16-20-0 fertilizer were applied to each pot during the winter.

Selfing in the greenhouse was effected by tripping the flowers and allowing them to self-pollinate. Since no insects likely to transfer pollen were present in the greenhouse, tripping the flowers either with a clean toothpick or by stroking the racemes with the fingers was sufficient to ensure self-pollination. In the field, however, all pods and opened flowers were removed and the remaining buds were covered with Kraft paper bags to exclude pollinating insects. The bags were manipulated at intervals of a few days to trip the flowers and the selfed seed was harvested at maturity.

To accomplish crossing, foreign pollen was collected with a toothpick and the flowers to be crossed were tripped on to it. In bulk crosses ten unrelated plants were used to supply the foreign pollen. In reciprocal crosses a flower from one plant was tripped on a toothpick, the pollen transferred to the stigma of a flower on the other plant by tripping it on to the same toothpick, and so on until the required number of flowers on each plant had been cross-pollinated. No emasculation was done for any of the crosses. It was assumed that a high percentage of crossing would occur without emasculation because of the high percentage of crossing occurring under field conditions of open-pollination. All crosses were made in the greenhouse.

A method of selecting for fertility was developed during the winter of 1942-43. The procedure was to self at least 30 and cross at least 30 flowers on each plant. The average number of seeds per flower selfed was taken as an index of self-fertility and the average number of seeds per pod from crossed flowers as an index of cross-fertility. Since a large number of flowers had to be treated, the work of labelling and recording was simplified by selfing or crossing exactly ten flowers on each raceme with all buds and older flowers removed. This also ensured the use of relatively fresh flowers. The self-fertility index was based upon the number of seeds per flower, since it took account of plants setting very few pods and differ-

entiated degrees of fertility among plants in which practically all flowers set pods. Any flowers failing to set pods because of mechanical injury would constitute an error in estimating the true self-fertility. In the material tested, practically all flowers set pods when cross-pollinated except when they were mechanically injured. Thus the number of seeds per pod, instead of the number of seeds per flower, was taken as the best estimate of true cross-fertility.

In field plot experiments single row plots were used with the rows spaced three feet apart. All were seeded in June 1943 at Snowden in the White Fox area with a Kemp V-belt seeder. The rate of seeding and length of row varied for different experiments depending on the amount of seed available. In most cases the rate of seeding was one seed for every 2.4 inches of row. This is a very low rate. Fortunately conditions for germination and emergence were good and generally satisfactory stands were obtained.

Wherever possible, because of the large number of treatments in the experiments and possible soil variability, the statistical design used for field plots was a simple lattice in duplicate as described by Hayes and Immer (25). Where the analysis of the results from this design was not applicable due to very large differences in yield or lack of replication from insufficient seed, simple averages were computed.

With the preceding discussion in mind, material and methods applicable to specific experiments will now be described.

Although over 80 per cent of cross-pollination in alfalfa has been reported (9, 34, 50), it was thought desirable to obtain further information on this point. Eight test plants with pure white flowers were used. This character is recessive to color. White-flowered plants when selfed or intercrossed produce only white-flowered progenies and, when crossed with colored-flowered ones, produce only colored-flowered progenies provided the colored parent is homozygous for color. The test plants were cloned and distributed in three fields at Saskatoon and allowed to set seed under open-pollination. Two of the fields yielded a poor crop and one a good crop of seed. The seed from the test plants was harvested in 1944 and grown and classified for flower color in 1945. In addition, two of the test plants were artificially crossed in the greenhouse. The resulting seed was grown and classified for flower color to test the efficiency of the greenhouse crossing technique.

The cross-fertility of inbred lines is important if they are to be combined in single crosses. Seed yield under good conditions for open-pollination is a test of cross-fertility and 48 first generation, 30 second generation and 20 third generation selfed lines were so tested. The seed yield data were determined from randomized test plots seeded at Snowden in 1943 and harvested in 1944. The plots were single rows ten feet long and spaced three feet apart. Where there was enough seed each inbred line was replicated four times. However, only three replications were possible for 14 lines, two replications for 14 lines and one plot each for 13 lines. Grimm and Ladak checks were included. Simple averages only were computed because yield differences were very great and the number of replications was not constant.

To determine the combining ability of individual alfalfa plants two groups were studied. One consisted of 13 plants selected for high seed yield from 13 of the best agronomic lines among the 33 inbred lines available. It is henceforth called the "inbred" group. Seven plants had been inbred for one generation and six for two generations. Five of the second generation plants traced back to one original parent but all others were unrelated. All had been selected for self-tripping and self-fertility. A second group, henceforth called "open-pollinated", was composed of 13 of the plants selected from fields grown from open-pollinated seed. Varietal origin was not a basis of selection but ten plants came from Grimm and three from Ladak stock. Six originated in fields setting a good crop of seed and seven from poor seed-setting fields. All were non self-tripping or nearly so, and all were highly cross-fertile according to the cross-fertility index, setting an average of over six seeds per pod when crossed. Plants varying in self-fertility from a low of 0.60 to a high of 5.22 (according to the self-fertility index) were chosen so that the relative value of self-sterility and self-fertility might be compared.

Within each of the above groups all of the possible 78 combinations were made in the greenhouse at Saskatoon during the winter of 1942-43. Since differences between a cross and its reciprocal might be expected if one parent was highly self-fertile and the other self-sterile, reciprocal crosses were made in both groups. The progeny of the cross, where the self-fertile plant is the pistillate parent, may contain many selfed plants which could be expected to reduce yields. In the inbred group certain plants afforded a comparison of the progenies of crosses between related plants with the progenies of crosses between unrelated plants. This comparison may be important in deciding whether or not the progenies of crosses between related plants are likely to be propagated among the progenies of single and double crosses. Where sufficient seed was obtained, each cross and its reciprocal was included in either of two seed and forage yield experiments at Snowden. One experiment contained crosses between plants in the inbred group and consisted of 57 crosses and their reciprocals and 18 crosses without reciprocals. Three crosses and their reciprocals failed to produce enough seed for testing. Six checks of Grimm and six of Ladak completed the 144 treatments required by the statistical design. The other experiment contained crosses between plants in the open-pollinated group and consisted of 63 crosses and their reciprocals and 14 crosses without reciprocals. One cross and its reciprocal failed to produce enough seed for testing. Two checks of Grimm and two of Ladak completed the 144 treatments required. Crosses between plants in the open-pollinated group set enough seed to plant single row plots 10 feet long with 50 seeds per row. Crosses between plants in the inbred group set much less seed and plots 5 feet long and 25 seeds per row were used. Both experiments were harvested for seed in 1944 and for forage in 1945. Seed yields were converted to pounds per acre and forage yields to tons per acre. The forage yields were based on green weights since the large number of treatments prevented dry-matter analysis because of a shortage of time and labor. Differences in maturity were not great and insufficient to affect the dry-matter content materially according to unpublished data at Saskatoon. A portion of the yield data was made use of in comparing the results from 1, 2 or 4 tester plants as a measure of combining ability.

EXPERIMENTAL RESULTS

Cross-pollination Studies

Clones from seven white-flowered plants were transplanted to each of three different alfalfa fields near Saskatoon. Fields No. 1 and No. 2 set a poor crop of seed and field No. 3 produced a heavy seed crop. A few clones did not survive transplanting but the remainder produced seed in 1944. This seed was planted in 1945 and the progenies classified for flower color. The data obtained are summarized in Table 1.

TABLE 1.—PERCENTAGE CROSS-POLLINATION OCCURRING IN ALFALFA FIELDS UNDER OPEN-POLLINATION AT SASKATOON AS DETERMINED BY GROWING AND CLASSIFYING FOR FLOWER COLOR THE PROGENIES OF SEVEN WHITE-FLOWERED PLANTS SCATTERED IN THE FIELDS

Strain number of white- flowered parent plants	Number of progeny plants classified for flower color			Percentage cross-pollination among progeny plants classified for flower color		
	Field No. 1	Field No. 2	Field No. 3	Field No. 1	Field No. 2	Field No. 3
S-41-25	38	6	233	11	17	81
S-42-60	48	101	1193	88	85	90
S-42-63	91	25	405	98	96	92
S-42-58	84	89		51	58	
S-42-59	66	29		91	100	
S-42-61	47	17		98	100	
S-42-62	64	369		92	92	

The data in Table 1 suggest that plant differences may be more important than location. Six of the seven plants reacted about the same when tested in different fields. However, the first one in the table, S-41-25, appears to have reacted differently in different fields although the progeny populations from fields No.'s 1 and 2 were small. This plant is relatively self-fertile and partially self-tripping which may account for the low percentage cross-pollination in fields No.'s 1 and 2 since good setting conditions in field No. 3 produced a progeny showing over 80 per cent cross-pollination. Plant S-42-58 showed only a moderate amount of cross-pollination in fields No.'s 1 and 2, as judged by its progenies, and it is unfortunate that it was not tested at No. 3. This plant was used in greenhouse crossing tests and it was noted that when used as a ♀ parent with different plants it varied in the amount of seed set. Some combinations gave a high number of seeds per pod and others set very few seeds. This fact may have some bearing on its performance in the field. In general, however, Table 1 confirms the results of previous writers (9, 34, 50) who found that alfalfa normally is cross-pollinated under field conditions.

Some information on the amount of cross-fertilization which occurred after artificial cross-pollination without emasculation was obtained from the progenies of white-flowered plants S-42-58 and S-42-60. When crossed with each other the progeny plants were uniformly white-flowered. Six progenies from S-42-58 and eleven from S-42-60, using them as the ♀ parent with different colored-flowered ♂ plants, were classified for flower color. Each progeny consisted of approximately 50 plants. Four

of the progenies from S-42-58 contained no white-flowered plants and the other two contained only a few plants (2 or 3) with white flowers. Similarly S-42-60 produced two progenies with no white-flowered plants and the other nine progenies contained only an occasional white-flowered plant. There is evidence that certain of the white-flowered plants were segregates from heterozygous colored-flowered plants since occasional white-flowered ones were found in the progeny of four different reciprocal crosses where a colored-flowered plant was the ♀ parent and either S-42-60 or S-42-58 the ♂ parent. It is concluded that crossing in the greenhouse without emasculation resulted in almost complete cross-fertilization.

Self- and Cross-Fertility Studies

During the winter of 1942-43 self- and cross-fertility ratings were obtained in the greenhouse on 107 of the plants selected for high seed yield in the fall of 1942. At least 30 flowers were selfed and 30 crossed on each plant. The number of seeds per pod was used as a measure of cross-fertility and the number of seeds per flower as an index of self-fertility. The data obtained are presented in the form of a scatter diagram in Figure 1.

A slight relationship between self- and cross-fertility is indicated by a highly significant correlation coefficient of 0.288. As seen from Figure 1, there was a wide range in self-fertility among the 40 plants considered highly cross-fertile, averaging over 6.00 seeds per crossed pod. Ten of this group of plants were highly self-sterile, setting less than one seed per flower selfed; while four were classed as highly self-fertile, setting three or more seeds per flower selfed. A possible criticism of the data is that, since no emasculation was used, the highly self-fertile plants may have given an erroneous value for cross-fertility due to their inherent self-fertility. As will be shown later by the data on crosses to determine combining ability, this is not a valid criticism for at least one highly self-fertile plant.

TABLE 2.—SELF- AND CROSS-FERTILITY OF THE PARENT PLANTS SELECTED FOR USE IN CROSSES TO DETERMINE COMBINING ABILITY. DATA OBTAINED IN GREENHOUSE AT SASKATOON DURING 1942-43

Open-pollinated Stock			Inbred Stock		
Strain No.	Average No. of Seeds		Strain No.	Average No. of Seeds	
	Per Flower selfed	Per pod crossed		Per Flower selfed	Per pod crossed
S-42-119	2.53	8.26	S-40-104-1	1.06	4.96
S-42-60	0.74	7.70	S-40-94-1	1.37	4.57
S-42-172	0.70	7.31	S-38-33-1-1	1.92	4.22
S-42-178	1.10	7.08	S-39-49-1	0.25	3.99
S-42-179	1.47	7.01	S-38-46-1-1	0.46	3.95
S-42-58	0.77	6.89	S-38-33-1-6	0.72	3.59
S-42-87	0.99	6.64	S-38-33-1-3	0.74	3.56
S-42-182	0.60	6.60	S-40-85-1	1.16	3.56
S-42-105	0.49	6.54	S-40-47-1	1.90	3.46
S-42-99	2.54	6.51	S-40-179-1	1.32	3.38
S-42-124	5.22	6.44	S-38-33-1-4	1.29	3.30
S-42-74	2.17	6.34	S-38-33-1-2	0.57	3.14
S-42-85	2.24	5.99	S-39-54-1	0.84	3.10
	1.66	6.89		1.05	3.75

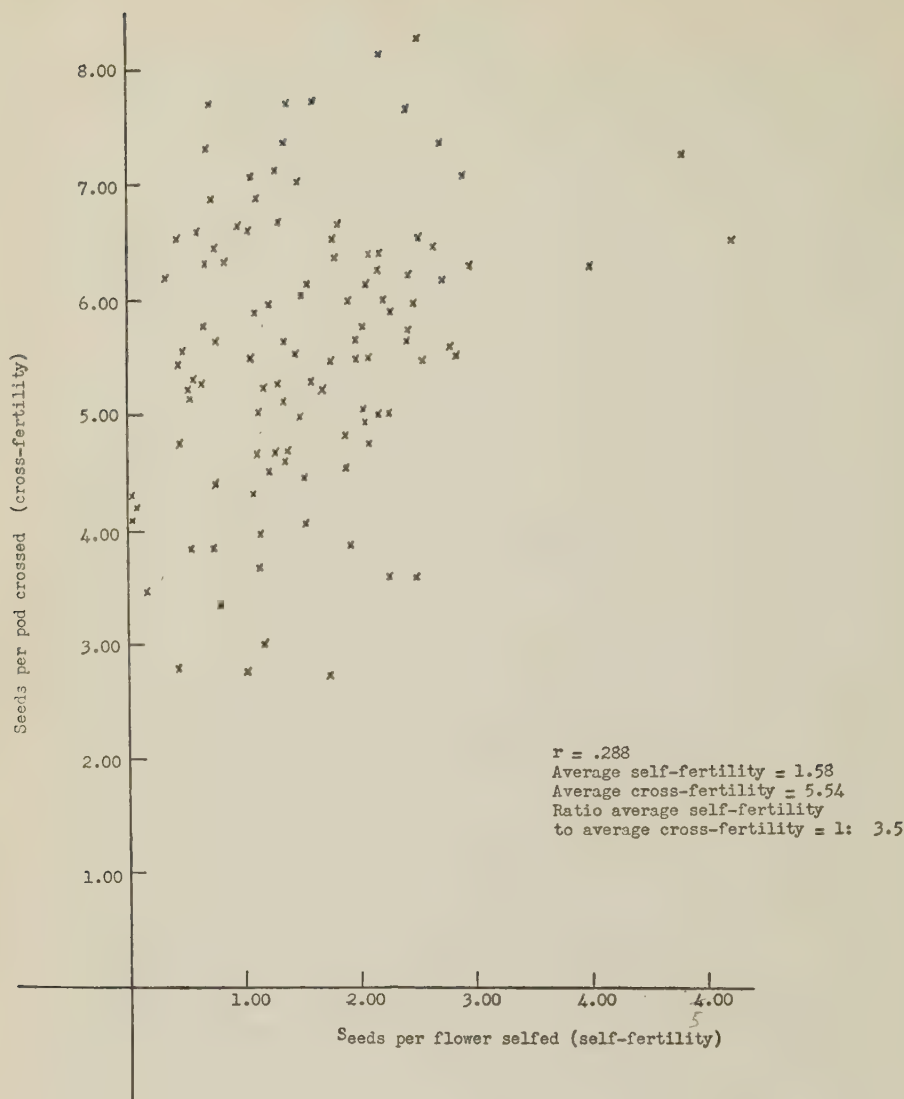


FIGURE 1. Self-fertility and cross-fertility and their relationship in 107 plants selected in the fall of 1942.

The above ratings on self- and cross-fertility were used to make the final selection of 13 parent plants to be used in crosses to determine combining ability among plants of open-pollinated stock. Similar fertility ratings were not used for the selection of the 13 inbred plants tested for combining ability but were obtained in the course of the studies. The actual fertility data for all 26 plants are presented in Table 2.

Among the selections from open-pollinated plants, as shown in Table 1, plants with high cross-fertility but showing varying degrees of self-fertility were chosen. Thus, S-42-124 was the most self-fertile plant tested while others, below an average of 1.00 seeds per flower selfed would be classed

as relatively self-sterile. The inbred selections showed a somewhat higher degree of self-fertility than might be expected. This is explained by the fact that the original selections and the parents of the second generation plants in the S-38 group were chosen for a high percentage of pod-setting. Thus there was selection for self-fertility as well as self-tripping.

The Seed Yield of Inbred Lines

Selfed seed from 98 different plants was seeded in seed yield plots in 1943 and the open-pollinated seed produced on the resultant progenies was harvested in 1944. Of the 98 progenies forty-eight were from first generation, 30 from second generation, and 20 from third generation progenies from selfing. Table 3 presents a summary of the yield data obtained.

TABLE 3.—FREQUENCY TABLE OF SEED YIELD DATA FROM 98 INBRED PROGENIES AND 8 CHECKS OF GRIMM AND LADAK. GROWN AT SNOWDEN 1943-44, HARVESTED IN 1944

Seed Yield—Lbs. per acre	0-9	10-19	20-29	30-39	40-49	50-59	60-69	100-200
1st generation lines	16	14	3	8	4	3		
2nd generation lines	24	5	1					
3rd generation lines	13	2	2		2		1	
Checks								8
Totals	53	21	6	8	6	3	1	8

No statistical analysis of the data in Table 3 is needed to show that differences in yield between inbred lines and between inbred lines and checks were very great. The average yield of the 48 first generation lines was 20 pounds per acre or about 13 per cent of the average yield of the check plots. Averages for the second and third generation lines were not calculated since they contained several families not equally represented and also because they had been selected for self-fertility.

The data in Table 3 show that it is possible to select inbred lines which, even in the third generation, will yield from 40 to 60 pounds of seed per acre when grown in field test plots where open-pollination prevails. This may be important in the production of commercial hybrids especially if the seed harvested proves to be the result of cross-pollination and if good combining lines can be selected.

Crosses Between Related Plants

Five second generation inbred plants in the inbred group were related and traced back to one selection S-38-33. Each of these five plants was selfed, intercrossed and outcrossed and the resulting seed planted in yield test plots. Fertility ratings based on seeds per flower crossed and selfed were available for the five parent plants. The seed yield data for the inbred progenies were taken from the data summarized in Table 3. The seed and forage yield data from the related and outcrossed progenies were taken from a test of combining ability described later in this paper. The above data are summarized in Table 4.

TABLE 4.—FERTILITY RATINGS OF FIVE RELATED PARENT PLANTS BASED ON SELFING, INTER- AND OUT-CROSSING, TOGETHER WITH SEED AND FORAGE YIELD DATA FROM THE PROGENIES THUS PRODUCED. YIELD DATA FROM PLOTS AT SNOWDEN, SEED HARVESTED IN 1944 FORAGE IN 1945

Parent Plants				Progenies				
Strain No.	Seeds per Flower			Seed Pounds per acre			Forage tons per acre	
	Selfed	Inter-crossed	Out-crossed	Selfed	Inter-crossed	Out-crossed	Inter-crossed	Out-crossed
S-38-33-1-4	1.29	2.46	3.17	4	54	345	2.50	4.09
S-38-33-1-1	1.92	2.14	3.98	40	123	290	4.07	4.89
S-38-33-1-2	0.57	2.18	3.04	21	76	254	2.81	3.45
S-38-33-1-6	0.72	1.55	3.23	19	69	210	3.04	3.82
S-38-33-1-3	0.74	1.72	3.37	28	68	170	3.14	3.97
Average	1.05	2.01	3.36	22	78	254	3.11	4.04
Av. in per cent of outcrossed	31	60	100	9	31	100	77	100

The intercrosses between related plants in this experiment would propagate any heterozygosity present at the end of the second generation of inbreeding and thus should be the equivalent of sib-crosses within a second generation inbred line. As shown in Table 4, the average number of seeds per flower resulting from intercrossing was only 60 per cent of that obtained from outcrossing. Similarly the average seed yield of progenies grown from intercrossed seed yielded only 31 per cent of the yield obtained from progenies grown from outcrossed seed, and even forage yields were down to 77 per cent. Comparable figures for seeds per flower selfed and seed yield of selfed progenies were 31 per cent and 9 per cent. Although the forage yields of the progenies from selfing were not recorded, observations suggested that they would have been lower than those from intercrossing.

The above results mean that in a second generation inbred line selfing and sibbing will produce considerably less seed, on a per flower basis, than will outcrossing. Furthermore, such seed from selfing or sibbing will produce plants that can be expected to produce much less seed than plants from outcrossing. Finally, the reduction in vegetative vigor as indicated by lowered forage yield should cause at least a partial elimination of plants from selfing or sibbing through competition with the more vigorous progeny of hybrid seed. This latter deduction is supported by the experimental results of Tysdal and Kiesselbach (49) who, in a comparison with Ladak, were unable to show a significant reduction in forage yield in the progeny obtained when a 50 per cent mixture of seed of a low-yielding inbred was mixed with Ladak seed. It appears probable that, in the production of a commercial alfalfa hybrid, considerable amounts of selfing and sibbing might be tolerated without materially affecting the yield of the hybrid.

Reciprocal Crosses

In the experiments set up to test combining ability within the inbred and open-pollinated groups, the seed from reciprocal crosses was planted in separate plots so that any differences which might exist could be measured. A comparison of reciprocals is presented at this point in order to assist in the interpretation of the data given later on combining ability. Since no emasculation was practised in crossing, the crossed seed might be expected to be contaminated with selfed seed in those cases where the ♀ parent was self-fertile. Further, if the proportion of selfed seed affects the yield of the progeny, then certain reciprocals should differ significantly in yield. Table 5 presents a summary of the data obtained.

TABLE 5.—AVERAGE YIELDS OF SEED AND FORAGE FROM THE PROGENIES OF RECIPROCAL CROSSES. PLOTS SEEDED AT SNOWDEN IN 1943. SEED YIELDS HARVESTED IN 1944 AND FORAGE YIELDS IN 1945

Parent Plants Strain No.	No. of Recip- rocal crosses	Seed Yield in Pounds per acre				Forage Yield in tons per acre			
		As ♀ Parent	As ♂ Parent	Differ- ence	L.S.D. at 5% Point	As ♀ Parent	As ♂ Parent	Differ- ence	L.S.D. at 5% Point
Inbred stock									
S-39-49-1	10	272	255	17	36	4.41	4.07	.34	.38
S-39-54-1	7	236	255	-19	43	4.04	4.41	-.37	.46
S-40-47-1	11	189	184	5	34	3.69	3.91	-.22	.37
S-40-85-1	8	252	276	-24	40	4.05	4.38	-.33	.43
S-40-94-1	11	246	242	4	34	4.31	4.01	.30	.37
S-40-104-1	11	197	238	-41	34	3.99	3.97	.02	.37
S-40-179-1	10	198	202	-4	36	3.51	3.38	.13	.38
S-38-46-1-1	8	246	216	30	40	3.46	3.42	.04	.43
S-38-33-1-1	8	296	284	12	40	4.96	4.82	.14	.43
S-38-33-1-2	6	267	248	19	47	3.60	3.52	.08	.50
S-38-33-1-3	5	142	156	-14	51	3.69	3.96	-.27	.54
S-38-33-1-4	7	343	321	22	43	3.82	4.06	-.24	.46
S-38-33-1-6	6	211	206	5	47	3.91	3.80	.11	.50
Open-pollinated stock									
S-42-58	7	297	263	34	31	4.76	4.83	-.07	.41
S-42-60	10	273	251	22	26	4.97	5.02	-.05	.34
S-42-74	10	216	221	-5	26	5.12	5.06	.06	.34
S-42-85	10	298	319	-21	26	5.15	4.92	.23	.34
S-42-87	6	217	192	25	34	5.72	5.37	.35	.43
S-42-99	8	182	257	-75	29	3.94	4.40	-.46	.38
S-42-105	9	280	253	27	28	4.05	3.95	.10	.35
S-42-119	12	308	310	-2	24	5.22	5.22	.00	.31
S-42-124	12	277	286	-9	24	4.93	5.03	-.10	.31
S-42-172	11	249	258	-9	25	4.61	4.81	-.20	.32
S-42-178	12	172	160	12	24	4.78	4.64	.14	.31
S-42-179	11	268	270	-2	25	5.10	5.04	.06	.32
S-42-182	8	247	233	14	29	6.29	6.30	-.01	.38

The data presented in Table 5 give little indication that artificial crossing without emasculation resulted in enough selfed seed significantly to affect the yield of the progenies. S-40-47-1 and S-38-33-1-1 were the most self-fertile plants in the inbred group (see Table 2) and the progenies of neither showed any real difference in the seed or forage yield of reciprocal crosses. In the open-pollinated group S-42-119, S-42-99 and especially

S-42-124 may be regarded as self-fertile plants. The progenies of S-42-119 and S-42-124 showed no real difference between reciprocals in either seed or forage yield. The progenies of reciprocal crosses with S-42-99 differ significantly in both seed and forage yield. The seed from this plant, however, germinated slowly and the resultant stands were later, and possibly thinner, as compared to the stands obtained from seed from other plants in the same group. It is thought that this condition, rather than a large proportion of selfed seed, was the cause of the differences for this line recorded in Table 5.

The data presented previously on percentage cross-pollination showed that crossing without emasculation gave almost complete cross-fertilization as far as parent plants S-42-58 and S-42-60 were concerned. Both of these plants were relatively self-sterile. The data on reciprocal crosses strongly suggest that the same conclusion may apply to highly self-fertile plants such as S-42-124. It may be objected that competition, as discussed in the section on crosses between related plants, might account for the similarity of reciprocal crosses rather than the absence of selfed seed. However, the rate of seeding in this experiment was very light and the rows were spaced 3 feet apart. Under these conditions weak plants are much less likely to be eliminated by competition than in broadcast seedings or closely spaced rows.

Determinations of Combining Ability by Crosses in all Possible Combinations

Since alfalfa is highly cross-fertilized under natural conditions, the final classification of selected parents requires that their cross progenies be tested. Crossing in all possible combinations and growing the resulting progenies under test is the most refined technique for this purpose. If carried out in full, an estimate of the yield of any one plant in combination with any other in the same group is provided. Tables 6 and 7 present seed and forage yield data for the open-pollinated and inbred groups after all crosses (as far as possible) within each group had been made and the crossed progenies grown in test plots:

In Tables 6 and 7 the upper triangle presents the data on seed yield and the lower triangle the data on forage yield. In each table and each triangle, each cell contains the average yield of four single plots for each of the reciprocals and the average yield of the reciprocals. For seed yield the upper figure in each cell is the average yield of the plant listed at the side of the table used as the ♀ parent with the line listed at the top, the second figure being the yield of the reciprocal cross. For forage yield the upper figure is the average yield of the plant listed at the top of the table used as the ♀ parent, the second being the reciprocal. Thus in Table 6, in the first cell of the upper triangle, 108 is the average yield in pounds per acre of the cross S-39-49-1 ♀ × S-39-54-1 and 81 is the yield in pounds per acre of the cross S-39-54-1 ♀ × S-39-49-1. The average of the reciprocals is 94 pounds per acre. In the first cell of the lower triangle, 4.26 tons per acre refers to the cross S-39-49-1 ♀ × S-39-54-1 and 3.50 tons per acre refers to the reciprocal S-39-54-1 ♀ × S-39-49-1 and 3.88 tons per acre is the average of the reciprocals. The average yield for all combinations given at the side and bottom of the table are averages of the averages of reciprocals for each plant.

TABLE 6.—1944 AVERAGE SEED YIELD IN POUNDS PER ACRE AND 1945 AVERAGE FORAGE YIELD IN TONS PER ACRE OF PROGENIES OF CROSSES BETWEEN 13 INBRED PLANTS, WITH GRIMM AND LADAK CHECKS AND APPROPRIATE SIGNIFICANT DIFFERENCES, SNOWDEN, SASK.

	S-39-49-1	S-39-54-1	S-40-47-1	S-40-85-1	S-40-94-1	S-40-104-1	S-40-179-1	S-38-46-1-1	S-38-33-1-1	S-38-33-1-2	S-38-33-1-3	S-38-33-1-4	S-38-33-1-6	Average seed yield all combinations
S-39-49-1		108 81 94	160 285 222	399 — 399	354 346 350	329 191 260	196 — 196	220 265 242	278 308 293	305 319 312	181 152 166	481 410 446	300 196 248	269
S-39-54-1	4.26 3.50 3.88		230 193 212	213 — 213	338 285 312	— 292 292	174 173 174	200 223 212	202 331 266	— 265 265	— 235 235	425 469 467	— 222 222	245
S-40-47-1	4.96 4.31 4.64	4.04 4.78 4.41		216 194 205	104 180 142	246 213 230	154 160 157	138 — 138	286 260 273	167 172 170	113 109 111	190 225 208	127 119 123	183
S-40-85-1	5.92 — 5.92	3.85 — 3.85	3.96 4.70 4.33		153 316 234	354 290 322	171 144 158	216 227 222	376 336 356	273 287 280	— 263 263	— 435 435	280 393 336	285
S-40-94-1	4.66 4.15 4.40	4.28 5.23 4.76	2.86 4.28 3.57	3.25 5.25 4.00		231 165 198	240 215 228	233 — 233	339 321 320	188 358 273	165 197 181	198 242 220	215 218 216	243
S-40-104-1	3.75 4.13 3.94	— 4.04 4.04	3.51 3.69 3.60	5.11 5.25 5.18	4.21 3.78 4.00		144 127 136	166 109 138	193 230 256	239 218 228	137 156 146	288 368 328	141 161 151	224
S-40-179-1	3.53 — 3.53	3.04 3.99 3.52	3.29 3.02 3.16	2.92 3.09 3.00	4.11 4.11 4.11	3.07 3.09 3.08		162 201 182	360 282 321	225 — 225	185 95 140	276 311 377	176 181 178	202
S-38-46-1-1	3.93 4.15 4.04	3.36 3.25 3.30	3.24 — 3.24	3.68 3.34 3.51	4.09 — 4.09	3.88 2.56 3.22	2.26 3.05 2.66		240 210 225	319 247 283	— 119 119	387 311 349	209 — 209	213
S-38-33-1-1	4.83 5.62 5.22	5.29 5.02 5.16	4.36 4.59 4.48	5.22 5.23 5.22	4.84 4.94 4.89	5.12 5.38 5.25	4.70 4.64 4.67	4.19 4.27 4.23		104 142 123	— 106 106	111 168 140	89 157 123	*290
S-38-33-1-2	3.64 3.24 3.49	— 3.39 3.39	3.29 3.63 3.46	3.68 4.41 4.04	3.24 4.48 3.86	3.87 2.94 3.40	2.86 — 2.86	3.42 2.79 3.10	4.50 3.53 4.02		— — —	30 31 30	— — —	*254
S-38-33-1-3	4.47 3.92 4.20	— 4.75 4.75	3.69 2.56 3.12	— 4.58 4.58	4.40 4.60 4.50	3.52 4.45 3.98	3.73 2.94 3.34	— 3.29 3.29	— 3.97 3.97	— — —	— — —	— 33 33	— — —	*170
S-38-33-1-4	4.79 3.83 4.31	4.74 4.33 4.54	3.17 3.75 3.46	— 5.12 5.12	3.78 3.58 3.68	4.12 4.64 4.38	4.11 3.39 3.75	3.73 3.23 3.48	3.99 4.30 4.14	1.60 — 1.60	— 2.32 —	— — 1.92	15 15	*345
S-38-33-1-6	4.82 3.74 4.28	— 4.02 4.02	3.36 3.75 3.56	3.81 4.98 4.40	4.38 3.61 4.00	3.41 4.05 3.73	3.04 3.33 3.18	3.36 — 3.36	4.65 3.68 4.16	— — —	— — —	1.92 — 1.92	— — —	*210
Average forage yield all combinations	4.32	4.14	3.75	4.43	4.16	3.98	3.40	3.46	*4.98	*3.45	*3.97	*4.09	*3.82	

* Yields of related crosses not included, thus average of 8 crosses instead of 12.

Grimm Checks	Seed		Forage		Least Significant Differences			
	199	123	4.79	4.36				
	147		4.22		Comparison			
	205		4.41					
	176		4.20		Seed		Forage	
	146		4.70					
Average		166		4.45	5% pt.		1% pt.	
Ladak checks	126		5.00		1 cross vs. 1 cross	113	149	1.21 1.59
	178		5.80		1 cross vs. 2 crosses	99	130	1.05 1.37
	139		5.22		2 crosses vs. 2 crosses	80	105	.85 1.12
	204		6.19		12 crosses vs. 12 crosses	33	43	.35 .46
	167		4.62		12 crosses vs. 8 crosses	37	48	.39 .51
	157		5.34		12 crosses vs. 6 checks	40	53	.43 .56
Average		162		5.36	8 crosses vs. 8 crosses	40	53	.43 .56
					8 crosses vs. 6 checks	43	57	.46 .61
Average all checks		164		4.90	6 checks vs. 6 checks	46	61	.49 .65

TABLE 7.—1944 AVERAGE SEED YIELD IN POUNDS PER ACRE AND 1945 AVERAGE FORAGE YIELD IN TONS PER ACRE OF PROGENIES OF CROSSES BETWEEN 13 PLANTS GROWN FROM OPEN-POLLINATED SEED, WITH GRIMM AND LADAK CHECKS AND APPROPRIATE SIGNIFICANT DIFFERENCES, SNOWDEN, SASK.

	S-42-58	S-42-60	S-42-74	S-42-85	S-42-87	S-42-99	S-42-105	S-42-119	S-42-124	S-42-172	S-42-178	S-42-179	S-42-182	Average seed yield all combinations
S-42-58		302 252 277	— 205 205	303 235 269	— 181 181	246 203 224	— 317 317	387 311 349	342 330 330	— 308 308	194 236 215	304 283 294	— 228 228	266
S-42-60	4.89 5.20 5.04		230 — 230	341 270 306	97 — 97	266 173 220	323 247 285	344 315 330	289 233 261	266 289 278	166 175 170	257 241 249	229 264 246	246
S-42-74	— 4.49 4.49	5.15 — 5.15		252 227 240	125 140 132	194 145 170	173 278 226	295 323 309	283 266 274	184 204 194	145 170 158	218 206 212	286 252 269	218
S-42-85	4.85 5.04 4.94	5.13 4.61 4.87	4.85 4.59 4.71	— — —	— 109 109	— 353 353	353 443 398	385 338 360	398 379 388	323 305 314	170 269 220	326 280 303	296 283 290	296
S-42-87	— 6.05 6.05	5.08 — 5.08	6.16 5.26 5.71	— 4.49 4.49	— — —	— 142 142	— — —	343 223 283	233 263 248	293 212 252	102 91 240	192 96 216	128 — 128	*171
S-42-99	4.10 4.30 4.20	4.54 4.00 4.27	5.03 4.53 4.78	— 4.57 4.57	— 3.97 3.97	— — —	187 274 230	230 319 274	239 299 269	223 308 266	60 150 105	246 246 216	216 — 216	226
S-42-105	— 4.83 4.83	4.11 4.34 4.22	4.01 4.23 4.12	4.53 4.33 4.43	— — —	3.19 4.15 3.67	— — —	330 323 326	281 284 282	216 158 187	140 171 156	314 306 215	215 — 215	*267
S-42-119	5.38 4.50 4.94	5.23 5.54 5.38	5.60 5.45 5.52	5.96 5.56 5.76	6.34 6.21 6.28	4.29 4.53 4.41	4.08 3.89 3.98	— — —	367 351 359	327 275 301	261 195 228	321 326 324	271 261 266	309
S-42-124	4.63 4.86 4.74	5.39 5.48 5.44	5.61 5.03 5.32	5.03 4.59 4.81	5.56 5.39 5.48	3.83 4.56 4.20	3.94 3.58 3.76	4.76 5.07 4.92	— — —	232 270 251	134 132 133	378 349 364	182 249 216	281
S-42-172	— 5.01 5.01	4.81 4.43 4.62	4.83 4.91 4.87	4.85 4.09 4.47	5.98 4.89 5.44	4.04 4.36 4.20	4.14 4.01 4.08	5.17 5.19 5.18	4.38 4.34 4.36	— — —	180 194 187	282 283 282	255 293 274	258
S-42-178	4.88 5.05 4.96	3.93 5.24 4.58	4.65 5.32 4.98	5.67 5.25 5.46	5.61 4.40 5.00	3.36 3.96 3.66	3.33 3.56 3.44	4.67 4.58 4.62	4.63 4.78 4.70	3.93 4.30 4.12	— — —	141 234 188	142 134 138	166
S-42-179	4.58 4.89 4.74	4.93 4.92 4.92	4.55 5.24 4.90	4.78 4.79 4.78	5.58 5.15 5.36	4.39 — 4.39	3.87 4.67 4.27	5.73 4.94 5.32	5.63 5.40 5.52	4.72 4.17 4.44	4.57 5.21 4.89	— — —	205 240 222	268
S-42-182	— 6.84 6.84	6.40 6.73 6.56	5.97 6.01 5.99	6.44 5.80 6.12	5.74 — 5.74	5.37 — 5.37	4.89 — 4.89	6.57 6.07 6.32	5.96 7.06 6.51	5.88 6.25 6.06	6.40 5.85 6.12	6.79 6.54 6.66	— — —	226
Average forage yield all combinations	5.06	5.01	5.04	4.95	*5.33	4.31	*4.15	5.22	4.98	4.74	4.71	5.02	6.10	

* Averages of 11 crosses instead of 12 due to failure of cross S-42-105 × S-42-87 and reciprocal.

Grimm checks	155	4.48	Least Significant Differences											
Average	103	4.10												
Ladak checks	154	5.80	Comparison											
Average	182	5.40												
										Seed		Forage		
										5% pt.	1% pt.	5% pt.	1% pt.	
Average	168	5.60	1 cross or check	vs.	1 cross or check	82	108	1.06	1.39					
Average all checks	148	4.94	1 cross or check	vs.	2 crosses or checks	71	94	.92	1.21					
			2 crosses or checks	vs.	2 crosses or checks	58	76	.75	.98					
			12 crosses	vs.	12 crosses	24	31	.31	.40					
			12 crosses	vs.	11 crosses	24	32	.31	.41					
			11 crosses	vs.	11 crosses	25	33	.32	.42					
			12 crosses	vs.	2 crosses or checks	45	58	.57	.75					
			11 crosses	vs.	2 crosses or checks	45	59	.48	.76					

In both tables the least significant difference (L.S.D.) values presented were calculated from the error of a single determination for the experiment. Thus for example in Table 7 the L.S.D. at the 5 per cent point (45 pounds and 0.57 tons) may be applied to the difference between the average of the Grimm checks and the final average for the crosses with S-42-58, since it is based upon the mean of eight Grimm plots (two replicated checks) and 48 plots of S-42-58 (12 replicated crosses). The extra plots involved where reciprocals occurred were disregarded to avoid a multiplicity of standard errors of a difference, although their inclusion would have increased the accuracy of the comparison. This increased accuracy is, however, not considered necessary in the interpretation of the data due to the large differences recorded between the checks and most of the crosses.

The most striking result of these experiments was the increase in seed yield. All averages except two in each group significantly outyielded the check varieties and in some cases the increase was close to or greater than 100 per cent. Since the yield of the check varieties is a measure of the average yield of unselected populations, the data are good evidence that the method of selecting the parent plants for seed yield (by pods set in the field, and seeds per pod in the greenhouse) was sound. The progeny averages of S-42-124 and S-42-119 (Table 7) are particularly interesting. The former was the most self-fertile plant tested (5.22 seeds per flower selfed) and the latter was classed as at least moderately self-fertile (2.63 seeds per flower selfed). Yet the averages of the crossed progenies were very high for seed yield and significantly greater than Grimm in forage yield. This suggests that certain self-fertile plants produced high-yielding progenies as noted by Tysdal and Kiesselbach (49) and that artificial crossing, even without emasculation, resulted in a high percentage of cross-fertilization. The performance of these plants, however, must be tested by growing progenies resulting from seed produced under natural conditions for open-pollination.

In assessing the value of self-fertile plants the possible effects of self-tripping should be kept in mind. Under favorable conditions at Saskatoon (i.e. high temperatures) all the flowers of certain plants may self-trip when no tripping will occur among the flowers of other plants. Various intergradations occur between these extremes. Stevenson and Bolton (45) present data to show that self-tripping results largely in selfed seed. Other workers have shown that various species of bees are largely responsible for tripping in alfalfa and that insect tripping is very likely to result in cross-fertilization (34). Consequently the amount of selfed seed produced by self-fertile plants should depend on the inherent tendency of a plant to self-tripping and the abundance of tripping insects. Highly self-tripping plants are likely to set a high percentage of selfed seed at any time since they will trip so readily that there will be little opportunity for insects to visit untripped flowers. Similarly non self-tripping plants can be expected to set a high percentage of cross-fertilized seed under all conditions since insect visitation must occur before seed is set. However, plants which are only partially self-tripping should behave differently according to the abundance of tripping insects. If few insects are present the portion of selfed seed resulting from self-tripping should represent a relatively high percentage of the total seed produced. If the insects are abundant the

portion of selfed seed should represent a relatively low proportion of the total yield. The performance of plant S-41-25 supports these conclusions. As noted in the discussion following Table 1, it was relatively self-fertile and partially self-tripping and showed a low percentage of cross-pollination when tested in a field where conditions for seed-setting were poor. Conversely, it showed a high percentage of cross-pollination when tested under good seed-setting conditions. Tysdal and Kiesselbach (49) warn against selecting plants which are high seed-producing under poor seed-setting conditions unless they are known to be low in self-fertility. It may be that the danger lies not so much in selecting self-fertile plants as in selecting those that are more or less self-tripping.

Although seed yield was the main objective in selecting the plants in the inbred group and the only criterion used to select the open-pollinated group, the forage yields in Tables 6 and 7 are of considerable interest. All the average forage yields of the progenies from inbred plants (Table 6) are significantly lower than the Ladak checks and seven of the thirteen are significantly lower than the Grimm checks. None is significantly higher than the Grimm checks. In the open-pollinated group (Table 7) none of the average forage yields of the progeny is higher than Ladak but four are not significantly different and those include the average for S-42-119 which produced the progenies with the highest seed yield. The averages of the progenies from ten plants significantly out-yielded Grimm in forage yield and the other three were not significantly different. These results are very promising from the standpoint of greatly increasing seed yield while maintaining or increasing forage yield.

Determinations of Combining Ability by the Use of Tester Plants

Crossing in all possible combinations and testing the resulting progenies is an excellent method to determine combining ability and probably should always be used in the final analysis of a small group of plants such as were described in the previous section. However, where many plants are to be tested it becomes economically impossible to make and test all combinations. If a very few plants could be crossed with each plant to be tested and the yields of the resultant progenies taken as an accurate measure of combining ability, similar to the top-cross method in corn breeding, then much time and labor could be saved. With this object in mind the seed and forage yield data of the progenies of plants in the open-pollinated group (Table 7) were examined.

Four high-combining tester plants were assumed to be testers and the remaining nine were assumed to be those to be tested. The progeny yield of a high-combining tester in combination with each of the nine plants to be tested was calculated. Then the average progeny yield of two high-combining testers in combination with each of the nine plants to be tested was computed and finally the average progeny yield of four high-combining testers in combination with each of the plants to be tested was calculated. It was necessary to have equal replication in order to measure the effect of different numbers of tester plants. This was accomplished by taking the progeny yield of a cross and its reciprocal (eight individual plot yields) where one tester plant was used. Where two tester plants were

being considered four plot yields were chosen at random from each and where four testers were considered two plot yields were chosen at random from each. To obtain a standard for comparison the average progeny yield in all possible combinations was calculated within the group of the nine plants being tested. In this case four individual plot yields were chosen at random where reciprocals occurred so that the standard for each of these plants was based upon 32 plots. An exception was necessary due to the failure of cross S-42-87 \times S-42-105 and reciprocal and the comparative yield for each of these two plants was based upon only 28 plots. The correlation coefficient was used to compare the standard yield with that obtained when one, two or four testers were used. A similar procedure was used to obtain comparisons assuming one, two or four low-combining plants as testers. The data for seed yield are presented in Table 8, and the data for forage yield in Table 9.

The results presented in Tables 8 and 9 are not conclusive. There is a fair indication that two or more plants are preferable to only one if testers are to be used. It would appear, also, that plants that are poor combiners are equally as good testers as plants that are good combiners. The considerable variability between the different correlation coefficients can be attributed very probably to the high standard errors of the experiments as reflected in the least significant differences reported in Table 7. These high standard errors are undoubtedly caused, at least in part, by the small plot size, low rate of seeding and large number of plots in each experiment. Where differences are small, conclusive results can scarcely be expected from these data and it seems unlikely that there would be large differences when different numbers of tester plants are used.

TABLE 8—AVERAGE PROGENY YIELDS IN POUNDS PER ACRE OF SEED FROM HIGH-COMBINING AND LOW-COMBINING GROUPS OF TESTER PLANTS CROSSED WITH NINE OTHER PLANTS AND COMPARED BY THE CORRELATION COEFFICIENT WITH THE AVERAGE PROGENY YIELDS OF THE NINE PLANTS CROSSED IN ALL POSSIBLE COMBINATIONS

Nine remaining plants crossed in all combinations		High-Yielding Testers			Nine remaining plants crossed in all combinations		Low-Yielding Testers		
Strain No.	Yield	S-42-119	S-42-119 S-42-124	S-42-119 S-42-124 S-42-85 S-42-179	Strain No.	Yield	S-42-178	S-42-178 S-42-74	S-42-178 S-42-74 S-42-99 S-42-182
S-42-58	249	342	319	279	S-42-58	289	217	216	240
S-42-60	223	325	272	269	S-42-60	255	167	210	202
S-42-74	197	315	290	252	S-42-85	300	219	221	312
S-42-87	140	280	258	216	S-42-87	190	92	108	153
S-42-99	208	275	257	273	S-42-105	294	162	198	229
S-42-105	236	328	306	332	S-42-119	313	227	268	261
S-42-172	237	297	258	286	S-42-124	313	136	215	245
S-42-178	171	227	193	211	S-42-172	262	187	196	240
S-42-182	210	269	253	278	S-42-179	296	185	214	195
"r" values*		0.657	0.601	0.842	"r" values*		0.671	0.890	0.726

* Significant values of "r" at the 5% and 1% points respectively for seven degrees of freedom are 0.666 and 0.798.

TABLE 9.—AVERAGE PROGENY YIELDS IN TONS PER ACRE OF FORAGE FROM HIGH-COMBINING AND LOW-COMBINING GROUPS OF TESTER PLANTS CROSSED WITH NINE OTHER PLANTS AND COMPARED BY THE CORRELATION COEFFICIENT WITH THE AVERAGE PROGENY YIELDS OF THE NINE PLANTS CROSSED IN ALL POSSIBLE COMBINATIONS

Nine remaining plants crossed in all combinations		High-Yielding Testers			Nine remaining plants crossed in all combinations		Low-Yielding Testers		
Strain No.	Yield	S-42-119	S-42-182 S-42-119	S-42-58 S-42-74 S-42-182 S-42-119	Strain No.	Yield	S-42-178	S-42-99 S-42-178	S-42-85 S-42-172 S-42-99 S-42-178
S-42-60	4.79	5.05	5.80	5.24	S-42-58	5.00	4.79	4.48	4.88
S-42-85	4.80	5.57	5.71	5.03	S-42-60	5.15	4.42	4.55	4.58
S-42-87	4.89	6.33	6.36	6.33	S-42-74	5.17	4.88	5.07	4.85
S-42-99	4.19	4.55	4.84	4.56	S-42-87	5.72	5.05	4.56	4.73
S-42-105	4.36	3.93	4.58	4.85	S-42-105	4.61	3.70	3.82	3.83
S-42-124	4.82	5.01	5.88	5.69	S-42-119	5.30	4.66	4.27	4.60
S-42-172	4.59	5.24	5.47	5.16	S-42-124	5.20	4.75	4.54	4.76
S-42-178	4.64	4.66	5.38	5.30	S-42-179	5.42	4.97	4.93	4.79
S-42-179	5.14	5.19	6.17	5.43	S-42-182	6.29	6.24	5.94	5.72
"r" values*		0.644	0.915	0.698	"r" values*		0.939	0.833	0.857

* Significant values of "r" at the 5% and 1% points respectively are 0.666 and 0.798.

DISCUSSION

As stated in the introduction, the main objective in this project was to determine suitable means of evaluating parental material for use in a breeding program. At the same time consideration was given to the best type and source of selections. Both the Grimm and Ladak varieties as well as both good and poor yielding fields were used as sources of selections. Plants open-pollinated in origin and plants inbred for one or two generations were represented and a deliberate effort was made to include plants varying widely in self-fertility.

The value of different varieties as source material is not conclusive as far as the results of these experiments are concerned, since Ladak was not well represented. All of the 13 plants in the inbred group and 10 of those in the open-pollinated group were Grimm selections and showed a wide range in self-fertility and combining ability. The three Ladak plants used in the open-pollinated group, S-42-74, S-42-85 and S-42-87 averaged 2.17, 2.24 and 0.99 seeds per flower selfed (see Table 2) and thus would be classed as intermediate to relatively self-fertile types. These three plants varied widely in combining ability for both seed and forage (see Table 7). The results of observations on spaced populations of Grimm, Ladak, Cossack, Viking and Turkestan, however, indicate that any one of these varieties is sufficiently variable to provide ample range for the improvement of seed and forage yield by selection.

All of the plants in the inbred group and seven from the open-pollinated group originated in poor seed-setting fields. The remaining six in the open-pollinated group came from fields producing high seed yields. Tysdal and his associates (47, 49, 50) emphasize the desirability of selecting in fields with good seed-setting in order to avoid self-fertility. The results

reported in this paper, however, show that several plants selected in poor fields proved to have high combining ability for both seed and forage yield (Tables 6 and 7; plants S-42-119, S-42-124, S-42-85 and S-38-33-1-1). All of these plants were moderately or highly self-fertile (Table 2) but, as pointed out in the presentation of results in Tables 6 and 7, the disadvantage of poor fields may lie in the danger of selecting self-tripping rather than self-fertile types. Furthermore, there may be advantages in selecting in poor fields if insect preference is a factor in seed yield. Vansell (53) reports a range from 11.0 to 38.3 per cent in the sugar content of alfalfa nectar when grown under wet and very dry conditions respectively, and about 10 per cent greater concentration in the Turkestan alfalfa as compared with Common alfalfa. If these differences occur and are inherited (as Vansell's data indicate) then it is to be expected that bees would prefer to work those plants having a high concentration of sugar in the nectar, and this may account for at least some of the heavily seeded plants found in poor fields. Carlson (10) found indications of resistance to lygus bugs in alfalfa and again such plants, if they occur, would be expected to produce a good yield of seed in fields where the general yield had been reduced by lygus damage.

In regard to open-pollinated versus inbred source material the results of this study support the conclusions of Tysdal and his co-workers (47, 48, 50). Plants very high in combining ability for seed yield were obtained in both inbred and open-pollinated groups. High combining ability for forage yield also was obtained particularly in the open-pollinated group (plants S-42-87 and S-42-182, Table 7). The occurrence of high seed yield with a decided increase in forage yield probably is fortuitous since there was no conscious selection for forage yield in the open-pollinated group. However, it is very encouraging since it is evident that the two characters can be combined. Since open-pollinated sources appear to be as productive of good hybrids as is inbred material it would be preferable to use open-pollinated or hybrid selections. Such selections yield seed heavily and this is important if the parent plants are to be vegetatively propagated for the production of commercial hybrids.

As a result of the experience gained in this study and the related literature a general procedure for selecting and testing alfalfa for seed and forage yield has been evolved at Saskatoon. Where disease-resistance is desired a mass population of disease-resistant plants is used. The plants are then examined for seed production. Those showing a high percentage of pod-setting in the fields are selected and at the same time the number of seeds per pod is taken into account, roughly, by noting the number of coils on the pods and by rubbing out representative pods. The selected plants are transplanted to the greenhouse during the month of September and further selected during the winter months. Self-tripping is checked by observing the tendency of the flowers to trip without manipulation and the self-tripping types are discarded. About 100 flowers are selfed and at least 30 are crossed on each plant to give self- and cross-fertility ratings. The self-fertility ratings are not at present used as a basis for discarding plants because it is felt that more information is necessary to determine the relative value of self-fertile and self-sterile types. Later in this discussion, a possible method of utilizing self-fertile, non-self-tripping types is con-

sidered. The selfed seed obtained from the self-fertility rating test is used for a test of the progeny produced by selfing to determine possible undesirable recessive genes. The cross-fertility ratings are used to discard plants low in cross-fertility. Before spring, each remaining plant, which presumably is cross-fertile and non-self-tripping, is cloned and set out in a polycross nursery such as described by Tysdal *et al.* (50). The seed obtained from the polycross nursery will be used to plant out seed and forage tests which should give a rough measure of combining ability such as is provided by the top cross method of testing corn hybrids. Finally the better plants as determined by their polycross progenies will be intercrossed and the various combinations studied to provide a final rating of the probable value of each in synthetic or hybrid combinations.

In the later stages of this work where many different combinations are being studied, the problem of proper isolation is important. At the present time fenced plots spaced about one-half mile apart have been established on native pasture land about twenty miles south of Saskatoon. This procedure is expensive if very many combinations are to be made. Two alternative methods will be tried out in the next year or two. The first is to use small screen cages at least $4 \times 4 \times 4$ feet in which small three-frame colonies of honey bees are liberated. It is known that honey bees confined in cages trip a large number of the alfalfa flowers present. The amount of cross-pollination they effect will be determined by the use of white-flowered tester plants. The other method for isolation is to establish small plots about 8 feet square at intervals about one-quarter mile apart along the railroad right-of-way in areas where alfalfa is not grown. This latter method eliminates the use of fencing since large animals are excluded by the railway fence. If this method works it may be preferable to the use of screen cages and honey bees since less expense would be necessary and conditions would closely approach those under which large scale isolations would be produced.

Once the parental plants have been selected and tested for combining ability the problem remains of how they should be utilized in the production of a superior crop of alfalfa. While no actual data on combinations of parental plants are presented in this thesis except for single cross progeny yields from artificial crosses, the increases in seed yields resulting from these combinations are so great that a discussion of future procedure is desirable. There appear to be several possibilities.

(1) *Increase of a single plant line:* This method was used in the production of Buffalo (21), Grimm 666 (30) and Viking (unpublished records at Saskatoon) and subsequent experimental tests have shown that these varieties have rather wide adaptation although yield of seed and forage has been only maintained or at best slightly increased as compared to standard varieties. The method may have possibilities where the original parent has been rigorously selected but up to the present it has produced no outstanding successes.

(2) *Synthetic varieties:* This is defined here as combinations of two or more plants propagated by seed through an indefinite number of generations. It would appear to be a promising procedure where the original parent plants have been thoroughly tested. The average progeny seed yield of all single crosses from S-42-119 more than doubled the average

of the check varieties (Table 7) and a similar value for S-38-33-1-4 (Table 6) again more than doubled the yield of the checks. Some of the progeny seed yields of the individual crosses from which these averages were computed produced as high as 447 pounds of seed per acre as compared to the check plot average of 164 pounds per acre (Table 6). The combination of a number of such plants as S-42-119 and S-38-33-1-4 to form a synthetic variety should result in a large increase in seed yield. Somewhat similar examples could be quoted for forage yield.

A difficulty in deciding upon basic material for a synthetic variety lies in determining the number of parent plants or lines that are to be combined. Tysdal *et al.* (50) presented theoretical data which suggest eight to sixteen plants of alfalfa as a minimum. In a later publication Tysdal and Kiesselbach (49) in discussing synthetic varieties of alfalfa, state that "Presumably, less reduction from close breeding will result if more than four high-combining clones are synthesized." Kinman and Sprague (28) conclude that "In general the most efficient number of lines to be included in a synthetic [of corn] will vary with the range of combining ability among the inbreds available as parents. On the basis of this study, four to six lines appeared to be the most efficient number." The conclusions of Kinman and Sprague are based upon diploid inheritance and Tysdal and his co-workers did not state definitely that their statements were not based on diploid inheritance. As noted previously, however, there is good evidence that the common cultivated forms of alfalfa are autotetraploids (37) and that autotetraploid ratios closely fit genetic data for at least certain characters (50). Therefore it is suggested that very few basic plants or lines are necessary for a synthetic variety of alfalfa especially where clones open-pollinated in origin are used. Such clones would, presumably, be hybrids or at least heterozygous and the progeny of a single cross would be the equivalent in heterozygosity of a double cross involving four inbred lines of corn. If autotetraploid inheritance is the rule then the theoretical heterozygosity of the resulting population would be much greater since Haldane (23) and Bartlett and Haldane (3) have calculated that to reach 50 per cent homozygosity a tetraploid must be selfed for 3.80 generations as compared to one generation for a diploid and, that with brother and sister matings, the values are 8.72 generations and 3.26 generations. At Saskatoon, synthetics of alfalfa are being started with as low as two open-pollinated plants as a base in order to compare them with synthetics having up to six or eight plants as a base.

(3) *The production of commercial hybrids:* A method of producing commercial hybrids has been outlined in detail by Tysdal and his co-workers (47, 49, 50) and it should be feasible, and preferable if synthetics do not prove satisfactory. One objection to their method is the amount of vegetative propagation required. This difficulty may be more apparent than real since considerable expense is justified if increases in seed and forage yield comparable to test plot data can be assured. The method of vegetative propagation described by White (56) may prove a valuable aid in this regard.

An alternative method of producing commercial hybrids involving much less vegetative propagation and utilizing self-fertile material is a possibility. Figure 2 illustrates a plan whereby this might be accom-

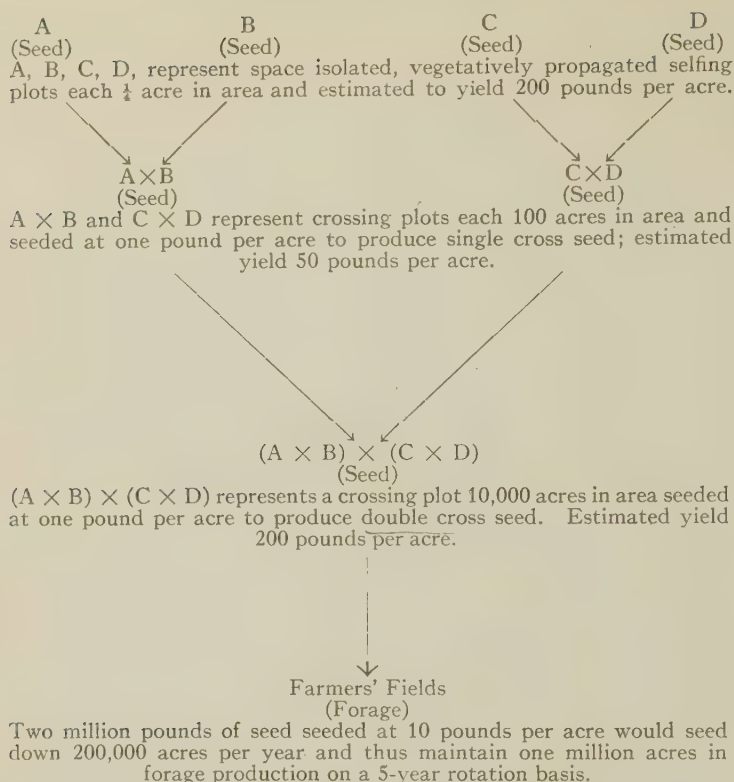


FIGURE 2. Illustration of the acreages required to maintain one million acres of double cross alfalfa.

plished. According to this plan only one acre of vegetative propagation is required to maintain the same acreage in forage production as 50 acres of vegetative propagation called for in the plan proposed by Tysdal *et al.* (50).

The plan outlined in Figure 2 assumes that four self-fertile plants can be found whose progenies from selfing will have high combining ability and that the resulting single and double cross progenies also will combine well. The yield of 200 pounds per acre assumed for the selfing plots should not be far out of line provided that plants with the self-fertility rating of S-42-124 can be used (see Table 2). The fields producing single cross seed will produce the seed on first generation selfed plants and therefore a yield of 50 pounds per acre only is assumed. This yield should also be possible according to the data in Table 3. The 200 pound per acre yield assumed for the fields producing double cross seed is a conservative estimate if the data reported in Table 7 can be taken as a guide. A major criticism of the plan is the use of self-fertile selections. Care in ensuring that the original parents were not self-tripping should reduce the proportions of selfed seed in the crossing plots. Then, too, Tysdal and Kiesselbach (49) were unable to show significant decreases in yield resulting from a 50 per cent mixture of seed of a low-yielding inbred with seed of the Ladak variety. Thus competition between inbreds and hybrids may largely eliminate the effect of rather large proportions of selfed seed and thus permit the utilization of self-fertile parental plants.

SUMMARY

1. The percentage of cross-pollination in alfalfa was determined by means of test plants recessive for white flower color. Under open-pollination the percentage varied from 11 to 100 per cent according to the test plant used and the amount of seed set in the field but was generally near 90 per cent or higher. Cross-pollination by artificial means but without emasculation gave almost complete cross-fertilization according to the two test plants used.

2. Self- and cross-fertility ratings were determined for over 100 plants. The self-fertility ranged from 0.02 to 5.22 seeds per flower selfed with an average of 1.58 and the cross-fertility varied from 2.67 to 8.26 seeds per pod with an average of 5.54. A highly significant "r" value of 0.288 indicated a slight relation between self- and cross-fertility.

3. Forty-eight first, 20 second and 20 third generation inbred lines were tested for seed yield under open-pollination conditions. The seed yield varied from 0 to over 60 pounds per acre. One third generation line yielded over 60 pounds per acre.

4. Crosses between second generation selfed plants originating from the same parents showed that the progeny of these related crosses were intermediate in seed and forage yield between comparable inbred lines and outcross progenies.

5. The progeny of reciprocal crosses, with very few exceptions, showed no significant difference between reciprocals. The crossed progenies of one highly self-fertile plant (S-42-124) were not significantly different in reciprocal crosses. This indicated that possible differences between reciprocal crosses, in the proportion of seed from self-pollination, either did not occur or were not important.

6. A group of plants open-pollinated in origin and a group of inbred plants were crossed in all possible combinations within each group. Yields of seed and forage were obtained from the crossed progenies and very great differences in the combining ability of parent plants were found.

7. The data obtained on combining ability for seed and forage yield of the open-pollinated group were used to examine the possibility of using one, two, or four high or low-combining tester plants in lieu of crosses in all combinations. Results were not conclusive but there were indications that tester plants may be useful in determining combining ability where large numbers of plants are to be tested.

8. The method used at Saskatoon to select parent plants suitable for breeding material is described. Selections for fertility are taken from either field populations or disease-resistant stock. These selections are observed for self-tripping and the self-tripping types discarded. Self- and cross-fertility ratings are obtained for each plant and progenies from selfed and crossed seed are grown. The selfed progenies are observed for possible undesirable recessive genes and the crossed progenies are tested for combining ability. To determine combining ability, progenies grown from polycross seed are used for the primary test and progenies grown from the seed from crosses in all possible combinations for the final selection.

9. An alternative plan to that proposed by Tysdal *et al.* (50) is outlined for the production of commercial hybrids in alfalfa. This plan suggests the possible use of non self-tripping, self-fertile plants to avoid, largely, the necessity for vegetative propagation.

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MUSTARD SEED OIL MEAL AND SUNFLOWER SEED OIL MEAL IN CHICK STARTER RATIONS¹

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Because of the present day shortage of animal proteins for poultry rations, the use of proteins from vegetable sources has become more widespread. These latter have been used to replace varying amounts of the individual or a combination of the animal proteins. Several of the newer vegetable proteins are sunflower seed oil meal and mustard seed oil meal. Pettit *et al.* (1) reported that under the conditions of their experiment, sunflower seed oil meal, when replaced on a protein equivalent basis, could be used as a complete substitute for meat meal in chick starters. As far as the author is aware, no information is available on the use of mustard seed oil meal as a protein supplement in chick starter rations.

An experiment was undertaken to compare mustard seed oil meal and sunflower seed oil meal and to add further information on the value of sunflower seed oil meal as a partial substitute for animal proteins.

MATERIAL AND METHODS

Eight randomized groups of 40 unsexed day-old New Hampshire chicks were used in this test. Group 1 constituted the control group (no vegetable protein), groups 2-4 and 5-8 were the experimental lots which were fed increasing amounts of sunflower seed oil meal and mustard seed oil meal, respectively (see Table 3). When one day of age, the chicks were weighed individually and each group was placed in a separate compartment of an electric battery brooder. At two weeks of age, the chicks were transferred to floor pens. Body weight was recorded bi-weekly for the eight weeks of the test. Feed and water were supplied *ad lib.*

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TABLE 1.—COMPOSITION OF THE BASAL MASH

Ingredients	Lb.
Ground wheat	37
Ground oats	20
Ground barley	17
Bran	5
Fish meal	2½
Meat meal (55%)	3½
Buttermilk powder	2
Vitagras	2
Alfalfa meal	1½
Limestone powder	½
Salt	½
Fish oil (1200A-200D)	½
Manganese sulphate	¼ lb. per ton
Riboflavin premix*
Total	92

* Sufficient added to supply 1700 micrograms of riboflavin per pound of feed.

TABLE 2.—CHEMICAL ANALYSIS OF THE BASAL DIET AND SUPPLEMENTS

Supplement	Moisture	Protein	Ash	Ca.	P ₂ O ₅
	%	%	%	%	%
Basal diet	7.7	19.5	4.96	0.80	1.37
Animal protein	6.7	52.3	16.37	4.66	5.95
Sunflower seed oil meal	6.3	37.4	6.92	0.44	2.84
Mustard seed oil meal	6.9	37.4	5.63	0.55	2.04

TABLE 3.—AMOUNTS OF SUPPLEMENTS ADDED TO THE BASAL DIET

Diet and group	1	2	3	4	5	6	7	8
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Basal diet	92.0	92.0	92.0	92.0	92.0	92.0	92.0	92.0
Animal protein	8.0	6.0	3.0	—	6.0	4.0	2.0	—
Sunflower seed oil meal	—	2.8	7.0	11.2	—	—	—	—
Mustard seed oil meal	—	—	—	—	2.8	5.6	8.4	11.2

The basal mash as well as the supplements were analysed for protein, calcium, phosphorus and ash. In order to maintain the same protein level in all groups of chicks, it was necessary to substitute greater amounts of the vegetable proteins because of their lower protein content. No other adjustment was made at this time since the relatively small amounts added would not materially affect the balance of the ration.

The basal ration is listed in Table 1.

From the above table it will be seen that the animal protein is in the proportion of $2\frac{1}{2}$, $3\frac{1}{2}$ and 2 for the fish meal, meat meal and buttermilk powder, respectively. When adding additional animal protein to the basal mash of the various groups, the above proportion was maintained. Table 2 presents the chemical analysis of the basal diet along with the supplements.

In order to maintain the same protein level in the mash of all groups of chicks, 1.4 pounds of vegetable protein was substituted for 1.0 pound of the animal protein mixture. Table 3 outlines the method of substitution.

DISCUSSION OF RESULTS

The unweighted means of body weight on a bi-weekly basis from hatching time to eight weeks of age are listed in Table 4. An examination of the original data indicated that the average body weight of the various groups was not affected by sex, since there was no significant difference in the ratio of males to females within each group. A comparison of the body weights of those groups on the different supplements indicates that mustard seed oil meal as fed under the conditions of this experiment is not a good substitute for animal protein. On the other hand, those groups receiving sunflower seed oil meal were quite comparable to the control lot. Table 5 summarizes the analysis of the weight data at eight weeks of age. The method of analysis employed was that of "Weighted squares of means" as outlined by Yates (3) and Titus and Hammond (2).

TABLE 4.—UNWEIGHTED MEANS OF BODY WEIGHT

Group	Age in weeks				
	0	2	4	6	8
	gm.	gm.	gm.	gm.	gm.
1	35.81	125.09	315.04	597.27	892.08
2	36.02	116.29	318.55	606.90	893.13
3	36.05	113.80	297.05	553.11	881.37
4	36.31	110.29	291.37	557.66	857.41
5	35.90	109.60	286.85	552.74	825.02
6	36.37	114.22	290.35	550.55	846.66
7	35.58	111.00	285.50	538.17	833.24
8	36.95	110.00	289.11	526.36	803.92

TABLE 5.—TESTS OF SIGNIFICANCE BETWEEN DIETS

Group	2	3	4	5	6	7	8
			%	%	%	%	%
1 vs.	†	—	5*	1**	1	1	1
2 vs.		—	5	1	1	1	1
3 vs.			—	—	5	1	1
4 vs.				5	—	—	1
5 vs.					—	—	—
6 vs.						—	1
7 vs.							—

† No significant difference.

* Significant at 5% point.

** Significant at 1% point

Groups 2 and 3 did not differ significantly in average final body weight when compared to the control group. Group 4 (basal plus 11.2 per cent sunflower seed oil meal) was significantly lower in weight than Group 1. This is not in agreement with Pettit *et al.* (loc. cit.) who found that up to 14 per cent of the sunflower seed oil meal, when substituted on an equivalent protein basis, could be used to replace meat meal. This represented a complete substitution for the meat meal. However, it should be pointed out that these authors fed bone meal and oyster shell *ad lib.* in addition to 1 per cent of each of these ingredients in the basal mixture. Our basal diet contained only $\frac{1}{2}$ per cent limestone powder and no bone meal. Reference to Table 2 indicates that the vegetable protein feeds are lower in both calcium and phosphorous than the animal protein mixture. It is not possible to state whether one or both of these minerals, if fed in greater amounts, would account for the differences noted herein. However, when not more than one-third of the total animal protein is replaced by sunflower seed oil meal, growth is satisfactory.

With respect to the groups receiving the mustard seed oil meal, the above statement may partly account for the significantly lower weights. Group 5 which received only 2.8 per cent mustard seed meal as well as 6 per cent of the animal protein mixture in the basal diet was significantly lower in body weight than either the control lot or Group 2 (2.8 per cent

sunflower seed oil meal plus 6 per cent animal protein). As the mineral content of both supplements was similar, the significantly lower weight cannot be explained entirely on this basis. Mustard seed oil meal as a feed ingredient is not unpleasant to taste (from the human standpoint) since the bitter taste of the mustard seed is removed with the extraction of the oil. No differences were noted in the feed consumption figures from the various groups; this would eliminate the possibility of the taste of the mustard seed oil meal being a factor which might account for the lowered rate of growth. No abnormalities were observed in any of the groups except one bird in Group 8 which developed perosis.

Mortality was very low throughout the test. The total loss of chicks was four—one from Group 1; one from Group 3, and two from Group 7.

SUMMARY

A basal diet containing 19.5 per cent protein was supplemented with varying amounts of either mustard seed oil meal or sunflower seed oil meal and fed to growing chicks. The results obtained warrant the following conclusions:

1. Sunflower seed oil meal is a satisfactory substitute in chick starters when replacing not more than one-third of the total animal protein mixture.
2. Mustard seed oil meal is comparable to sunflower seed oil meal when analysed for protein, calcium and phosphorus.
3. The substitution of mustard seed oil meal in quantities as small as 2.8 per cent (equivalent to 2 per cent animal protein mixture) results in significantly lower body weight at eight weeks of age.
4. The poor growth resulting from the feeding of mustard seed oil meal cannot be explained fully on the basis of its calcium and phosphorus content.

ACKNOWLEDGMENT

The author wishes to thank Dr. E. Y. Spencer of the Department of Chemistry, University of Saskatchewan, for the chemical analysis of the feeds used in this test.

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GERMINATION OF BORON-DEFICIENT PEAS

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Boron deficiency in peas appears only recently to have come to the attention of growers. It was suggested to the writer as a possible explanation of anomalous germination behaviour in certain lots of peas by N. G. Lewis, District Analyst, Division of Plant Products, Calgary, in the spring of 1947 when he sent three samples of peas (*Nos.* 76-3026, 76-747 and 76-359) of the variety *Resistant Surprise* grown in Creston, B.C. on a soil known to be deficient in boron, to the Seed Research Laboratory for study. These samples, as well as all others used in this study, had been treated with Spergon.

A review of the literature revealed little bearing directly on the subject of this investigation although the general literature on boron requirements in plants is very considerable. The University of Kentucky Experiment Station (3) lists peas among those plants having a fairly high boron content. This appears to be borne out in a table given by Dennis (1) quoted from Bertrand and de Waal, which shows peas as having 21.7 mg. of boron per kg. dry matter among a list of plants ranging from 2.3 mg. to 94.7 mg.

However, Löhnis (5), comparing her results with those obtained by Bertrand and de Waal, finds that agreement, while good in many cases, notably in the *Gramineae* in which the boron content is low, is not uniformly good in the other species tested. In peas, her figures are 23.3, 10 and 3.3 mg. B per kg. dry matter. These different values are associated with differences in variety, having been all grown in the same experimental field. Other values obtained by Bertrand and de Waal are much higher which Löhnis suggests may be due to a difference in the boron content of the soils in which the plants were grown.

Schropp and Arenz (6) found that in sand culture, in which rape had been grown the previous year after an application of about 8.8 kg. borax per hectare, peas (variety not named) produced a lower yield by 9.9 per cent than in the control without borax. On the other hand, Leroux (4) using a soil exceptionally rich in organic matter (organic carbon about 4.5 per cent) but on which chemical fertilizers had not been used, reported increased yields of the variety *Early d'Annonay* with boron over the control. The increase was less, however, with 5 mg. of boron (as borate per kg. dry soil) than with 2 mg., the increases in weight of stems, leaves, pods and seeds collectively being 0.24 per cent and 5.5 per cent, respectively for the two boron rates.

Löhnis, in her extensive review of literature, mentions peas as one of the species for which other workers have reported conflicting results as to its sensitiveness to boron deficiency. She reports increases in dry weight of from 25 per cent to 118 per cent from the use of tap-water containing .0004 mg. boron per litre over distilled water in culture solutions. The lowest increase was given by the variety *American Wonder* which, she concludes, is highly sensitive to boron deficiency.

¹In charge of Seed Research.

It appears, then, that peas may be widely variable in boron content, and so, presumably, in their boron requirements, and in sensitivity to boron deficiency.

Nothing has been found in the literature on the germination of peas produced in boron-deficient areas.

CHARACTERISTICS OF BORON-DEFICIENT PEAS

Boron-deficient peas appear to the eye entirely normal. When germinated on a standard laboratory medium such as sand, however, a characteristic type of abnormal sprout is produced. Such sprouts are somewhat pale, stunted, and lack the recurved plumular bud typical of normal sprouts. Moreover, the bud itself fails to develop. The root system, however, appears normal. Figures 1 and 3 show tests which have produced such abnormal sprouts. In the later stages, and occasionally with quite small sprouts, a type of abnormality is sometimes seen in which multiple branching occurs, the branches being stunted and somewhat thickened. Multiple branching is also a symptom of boron deficiency in alfalfa, according to Dunklee and Midgeley (2).

In any one lot of peas grown on boron-deficient soil there are a few which develop perfectly normal sprouts as well as some intermediate types which are hard to classify. This suggests that the plant conserves the boron present in the original seed by storing most of it in one or two of the peas produced.

THE SEED ANALYST'S PROBLEM

Abnormal sprouts produced in the germination test are not counted as "germinated" under the Canadian Rules for Seed Testing and the same procedure is followed under the Rules of the Association of Official Seed Analysts and of the International Seed Testing Association. However, when boron-deficient peas are grown on normal soils, a satisfactory stand is obtained, while when such seed is used on boron-deficient soils, crop failure results. Since the seed analyst does not know under what conditions a given lot of seed will be grown, the interpretation of boron-deficiency abnormals becomes a difficult problem.

PRELIMINARY TESTS

When the seed was first received from Calgary the cause of the abnormal sprouts was not clear. Boron deficiency was suspected, as were also the possibilities that the amount of water in the tests was not optimal and that the sand used in the Calgary laboratory was unsatisfactory. In the Seed Research Laboratory a sand and sawdust medium is often used, particularly when it is desired to supply a high moisture level to the seeds. The peas were accordingly tested in this medium by the standard procedure. The three samples gave 87 per cent, 73 per cent, and 75 per cent, respectively of perfectly normal sprouts with a few abnormals which were not characteristic of the type described above.

TABLE 1.—GERMINATION PER CENT OF "BORON-DEFICIENT"
PEAS TESTED IN SAND AND SAWDUST

Sample	Normal sprouts	"Boron- deficient" abnormals	Other abnormals	Dead
76-3255	88	0	4	8
76-3256	80	0	6	14
76-3258	86	0	2	12
76-3262	66	0	10	24
76-3282	98	0	2	0
76-3284	82	0	6	12

About the same time that these three samples were sent, six others were sent by Mr. Lewis and, as well, a liberal supply of the sand used in Calgary. These were tested in sand and sawdust by the standard procedure and also in the Calgary sand, using a technique described in detail by Mr. Lewis, which has proved satisfactory for normal peas. The Calgary-method tests on these samples were so poor that a formal count was not made. Only a very small percentage emerged and those that did were typical of those described above as being due to boron deficiency. The tests in sand and sawdust are given in Table 1.

The results were so clear-cut that large replicates were not considered necessary, results in most cases being based on two replicates of 25 seeds each.

It should be mentioned that, apart from the difference in the germination medium, the tests also differed in amount of water used. By the Calgary method 6.8 cc. of water were added to 100 cc. of dry sand, as compared with 32.2 cc. of water in 100 cc. of sand and sawdust.

PATHOLOGICAL EXAMINATION

The possibility that pathogens might be responsible for the abnormality was considered. W. C. Broadfoot, Division of Botany, Science Service, kindly plated out a number of seeds of the three samples first received and of the first three samples in the tabulation above. According to his report none of the fungi isolated could be considered as causing such abnormal seedlings.

STUDIES WITH SUPPLEMENTARY BORON

Two possible explanations suggested themselves for the great difference in the results obtained with sand and with sand and sawdust. The first was that the moisture relations in sand and sawdust were more suitable for these lots of peas and the second, that the sand and sawdust supplied something that was lacking in the sand.* Because boron-lack had been suggested as causing the abnormal sprouts, this was the obvious substance to test. Accordingly, Project RL175, Expts. A, B, C and D were set up according to the following plan, using sample *No. 6229* (of which 76-3026 was a sub-sample).

* Experiments K and K¹, reported later, provide checks on the sand used in the Seed Research Laboratory. Without added boron it produced the same kinds and proportions of abnormal sprouts as did the Calgary sand.

Project RL175

Expt.	No. seeds	cc. water per 100 cc. sand	cc. 0.01% borax soln. per 100 cc. sand
A	2 × 25	6.9	
B	2 × 25		6.9
C	2 × 25	14.4	
D	2 × 25		14.4

Calgary sand was used in all cases. The test was completed on June 25, 1947 and repeated at a later date. The results are given in Table 2.

TABLE 2.—GERMINATION PER CENT OF PEAS BELIEVED TO BE DEFICIENT IN BORON WITH AND WITHOUT SUPPLEMENTARY BORON AT TWO MOISTURE LEVELS

Expt.	Date completed	Moisture level	Boron added	Normal	"Boron-lack" abnormal	Other abnormal	Dead
A	25/6	6.9	No	16	70	0	14
	15/7	6.9	No	4	78	14*	4
B	25/6	6.9	Yes	86	0	6	8
	15/7	6.9	Yes	82	0	10	8
C	25/6	14.4	No	8	72	0	20
	15/7	14.4	No	2	72	8	18
D	25/6	14.4	Yes	74	0	16	10
	15/7	14.4	Yes	82	0	10	8

NOTE.—70 per cent is at the 5 per cent level of significance for a test of 86 per cent.

* Just started germination so a little difficult to tell whether they should be classed as "Boron-lack" abnormal or other abnormal.

The results of the series completed on 15th of July, 1947 are shown in Figures 1, 2, 3 and 4.

As a check on whether the sodium in the borax might have been the factor responsible for the elimination of the abnormal sprouts referred to as "boron-lack", four lots of twenty-five seeds were tested using a solution of sodium bicarbonate equimolar to the 0.01 per cent borax solution with respect to sodium. The results are given in Table 3.

TABLE 3.—GERMINATION PER CENT OF PEAS USING SODIUM BICARBONATE SOLUTION EQUI-MOLAR TO THE 0.01% BORAX SOLUTION WITH RESPECT TO SODIUM

No. seeds	Normal	"Boron-lack" abnormal	Other abnormal	Dead
100	8*	73	11	8

* Three of these were border-line sprouts, showing boron-lack effects.

It is quite clear from these results that lack of boron, rather than unsuitable moisture conditions, is the cause of the type of abnormal sprout produced.

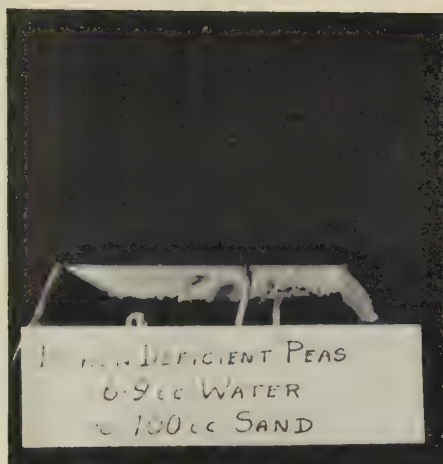


FIGURE 1

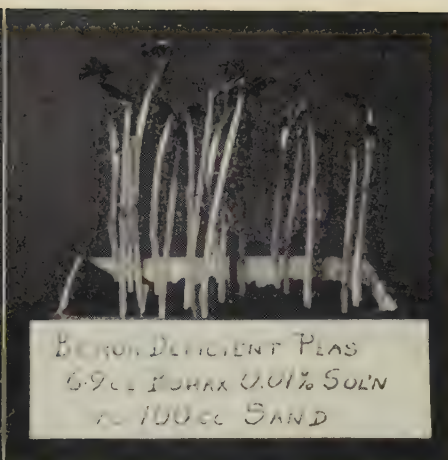


FIGURE 2

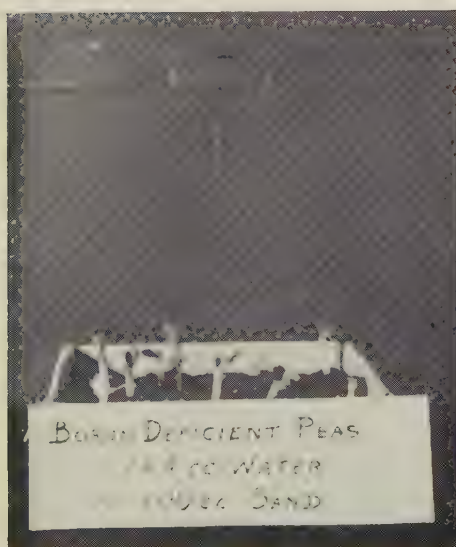


FIGURE 3



FIGURE 4

The only significant difference in the sprouts produced at the two moisture levels is in their lengths which are greater at the higher moisture level.

For the seed analyst, testing samples suspected of producing boron-deficiency abnormalities in a mineral medium known to be boron-free, with and without supplementary boron, provides a positive means of identifying this type of abnormal sprout.

THE GROWER'S PROBLEM

From the grower's point of view the problem may readily be solved by application of boron to the soil lacking this element. However, a stock of peas, otherwise highly desirable, may be lacking in boron. It seemed of interest to study the possibility of treating the seed to supply the deficiency.

SEEDS SOAKED IN BORON SOLUTION

For each experiment, fifty seeds of Sample No. 6229 were set to soak in 35 cc. of water or borax solution in a 10 cm. Petri dish. They were then planted with or without further treatment in pure quartz sand (such as is used in the Seed Research Laboratory), moistened with 11.5 cc. water to 100 cc. sand, using two replicates of twenty-five seeds each.

PRELIMINARY TESTS

Two soak periods were used, seventeen hours and seven hours, and the seeds were planted immediately after draining without drying. The seeds were soaked in water and in 0.01 per cent and 0.1 per cent borax solutions. Results are given in Table 4.

TABLE 4.—BORON-DEFICIENT PEAS SOAKED IN WATER, 0.01% AND 0.1% BORAX SOLUTIONS WITHOUT DRYING BEFORE BEING PLANTED. GERMINATION PER CENT

Expt.	Soak period	Soaked in	Normal	Boron-lack abnormal	Other abnormal	Dead
E	17 hrs.	Water	14	36	0	50
		0.01%	60	2*	10	28
		0.1 %	40	2	14	44
F	7 hrs.	Water	24 + 14†	30	12	20
		0.01%	58	0	16	22
		0.1 %	58	0	12	30

NOTE.—41 per cent is at the 5 per cent level of significance for a test of 60 per cent.

* Not typical boron-lack abnormal as described above but showing unnatural branching similar to later stages of boron-deficient sprouts.

† These sprouts would be called normal in a regular germination test but actually showed boron-deficiency effects.

The sprouts produced by the 0.01 per cent boron treatment were the best, averaging 6 inches or more, compared to 3½ inches to 4½ inches in the 0.1 per cent boron treatment, and 3 inches to 4 inches for the best sprouts in the water treatment.

Comparing these tests with the earlier tests in which supplementary boron was added to the sand, it appears that some injury has resulted from the soaking treatment in the boron solutions. To determine whether such injury could be avoided by partial or complete drying, Expts. G, H, I and J were carried out.

FURTHER TESTS ON SOAKED SEEDS

Experiments G and H were soaked for seven hours and I and J for seventeen hours in water and in 0.01 per cent and 0.1 per cent borax solutions. Before planting, G and I were thoroughly surface dried with a paper towel and H and J dried to constant weight at room temperature in an air-stream from a fan. Results are given in Table 5.

TABLE 5.—BORON-DEFICIENT PEAS SOAKED IN WATER, 0.01% AND 0.1% BORAX SOLUTIONS AND DRIED BEFORE PLANTING. GERMINATION PER CENT

Expt.	Soak period	Soaked in	Drying	Normal	Boron-lack abnormal	Other abnormal	Dead
G	7 hrs.	Water	Surface	24	46	6	24
		0.01%	"	68	0	16	16
		0.1 %	"	64	0	12	24
H	7 hrs.	Water	Constant weight	6	58	6	30
		0.01%	"	64	0	12	24
		0.1 %	"	66	0	14	20
I	17 hrs.	Water	Surface	28	16 + 10*	14	32
		0.01%	"	56	0	20	24
		0.1 %	"	52	0	20	28
J	17 hrs.	Water	Constant weight	0	64	12	24
		0.01%	"	62 + 8†	2	12	16
		0.1 %	"	52	2	20	26

NOTE.—51 per cent is at the 5 per cent level of significance for a test of 70 per cent.

* Not quite typical of boron-lack abnormal but showing unnatural branching similar to later stages of boron-deficient sprouts.

† These sprouts would be called normal in a regular germination test but actually showed some branching similar to later stages of boron-deficiency.

These results show no significant improvement over the lots which were soaked but not dried. The percentages of normal sprouts are not so high as in the tests in which boron was added to the sand and the "other abnormals" and dead seeds in the lots soaked in boron solution are considerably higher.

In view of the injury sustained by seeds soaked in boron solution, the feasibility of dry treatment was explored.

DRY TREATMENT OF SEEDS WITH BORAX

The same Sample, No. 6229, was used in these tests. Two replicates of twenty-five seeds were used in each case, as before, and the same test procedure was followed. Samples were (1) untreated, (2) dusted with one part of borax diluted with nine parts of talc and (3) dusted with pure borax. The seeds were placed in an Ehrlenmeyer flask with excess of dust and shaken until no more dust appeared to be adhering. Excess dust was then removed by shaking gently over a screen. Experiment K was planted immediately and experiment L after shaking the seeds mechanically over a screen at four excursions per second for five minutes. The results are given in Table 6.

TABLE 6.—BORON DEFICIENT PEAS UNTREATED AND TREATED DRY WITH 1/10 BORAX DUST AND PURE BORAX POWDER. GERMINATION PER CENT

Expt.	Borax dust	After treatment	Normal	Boron-lack abnormal	Other abnormal	Dead
K	None	None	22	64	6	8
	1/10	"	88	4*	4	4
	Pure	"	88	4*	4	4
L	None	Shaken 5 mins.	22	66	4	8
	1/10	"	74	6*	6	14
	Pure	"	82	4	8	6

NOTE.—73 per cent is at the 5 per cent level of significance for a test of 88 per cent.

* Not fully typical boron-deficiency abnormals.

It is evident from these results that dusting with borax is effective in preventing the development of boron-deficiency abnormals without injury to the seed even when pure borax is used. There is a suggestion that rough handling, as exemplified by the shaking treatment, may reduce the germination of the seed but statistically, the differences are not significant. There is no appreciable increase in the number of boron-deficiency abnormals as a result of the shaking.

In order to establish the significance of any differences, the experiment was repeated on a much larger scale, 8×25 seeds being used for each method. The results are given in Table 7.

The general level of germination of Expts. K¹ and L¹ is somewhat lower than of Expts. K and L but the trend is mostly the same. However, in L¹ the results of 1/10 borax and pure borax have been reversed. The only significant differences are those between borax treated and untreated tests. We must conclude, therefore, that treatment with dry borax powder, whether diluted to 1/10 or used pure, is effective in overcoming boron-deficiency in peas as far as germination is concerned.

TABLE 7.—BORON-DEFICIENT PEAS UNTREATED AND TREATED WITH 1/10 BORAX DUST AND PURE BORAX POWDER. (REPETITION OF EXPERIMENTS K AND L) GERMINATION PER CENT

Expt.	Borax dust	After treatment	Normal	Boron-lack abnormal	Other abnormal	Dead
K ¹	None	None	5.5	75.0 + 1.5*	10.5	7.5
	1/10	"	78.5 + 0.5†	2.5*	12.0	6.5
	Pure	"	78.5	1.0 + 3.5*	9.5	7.5
L ¹	None	Shaken 5 mins.	10.0	68.0 + 3.5*	11.0	7.5
	1/10	"	82.0	2.0 + 3.0*	7.5	5.5
	Pure	"	74.0	3.0*	14.5	8.5

NOTE.—74 per cent is at the 5 per cent level of significance for a test of 82 per cent.

* This would be called normal in a regular germination test but actually showed some boron-lack effect.

† Not fully typical boron-deficient abnormals.

SUMMARY

Nine samples of Resistant Surprise Peas, which appeared perfectly normal but which were grown in a boron-deficient area in Creston, B.C., were tested in various media and one of these was extensively tested with and without added boron. The addition of boron completely overcame the special type of abnormal sprouts associated with boron deficiency and the use of tests with and without added boron provides a positive means of identifying this type of abnormal sprout. The boron may be added in the form of a 0.01 per cent borax solution to moisten the germination medium or by dusting the seed with borax either pure or in 1/10 dilution with talc. For laboratory technique the use of the borax solution is probably to be preferred.

Certain sawdusts*, admixed with sand, also correct the condition, so that in using the sand-sawdust technique, the possible presence of this deficiency in peas may be concealed. This is a defect in the method which was not suspected at the time when it was proposed.

It is apparent from the literature that peas grown on soil not lacking in boron are sensitive to boron deficiency when planted in media deficient in this element. Marked increases in yield result from extremely small quantities of boron although different varieties differ widely in their response. The question of the minimum quantity of boron needed to bring about normal development and ripening of the seeds and the further growth of such seeds on boron-deficient soil does not seem to have been studied. It is reasonable to suggest that the Creston soils in which the peas of this investigation were grown were of a type in which such a minimum quantity of boron was present, since, on information received from Mr. Lewis, normal crops were obtained in those areas, and yet the seeds produced in the main were incapable of producing normal plants without added boron.

To pursue the study further is beyond the scope of this paper and of a seed laboratory. Nevertheless, the experiments which show the beneficial effect of dusting with borax do carry the investigation a stage further. (It should be mentioned that adherence of the borax was probably facilitated by the presence of Spergon.) Since very minute doses of boron are needed by the pea for normal development, it is possible that borax dusting of the seed would supply sufficient for the needs of the plant.

ACKNOWLEDGMENT

Sample No. 6229 which was the same as sample No. 76-3026 was kindly supplied by G. E. Nutile, Seed Analyst with Associated Seed Growers Incorporated. The author is also indebted to W. H. Wright and N. G. Lewis for reading the manuscript and suggesting certain changes.

* According to Mr. Lewis, the spruce sawdust available to the Calgary laboratory provides an excellent medium for normal peas but fails to correct the boron defect.

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ERADICATION OF POISON IVY (*Rhus radicans* L.)

III. FURTHER PRELIMINARY RESULTS WITH 2,4-DICHLOROPHENOXY-ACETIC ACID FORMULATIONS, AMMONIUM SULFAMATE, AND SODIUM CHLORATE¹

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In a previous paper (1), which gave the results obtained from applications of 2,4-D (2,4-dichlorophenoxyacetic acid) to poison ivy in 1945, it was reported that the best control was obtained when 2,4-D was applied to young plants in June and in concentrations of 1000 ppm or greater. Preliminary results, on the effects of 2,4-D acid, its ammonium salt, its triethanolamine salt, and its methyl ester on poison ivy, are now available from the 1946 treatments. For comparison are given the results obtained with 1 pound per gallon of ammonium sulfamate and of sodium chlorate. A final report will be published when complete results are available.

The method of application, location of plots, and preparation of the 2,4-D acid spray were similar to those described previously (1). The ammonium salt, the triethanolamine salt, and the methyl ester of 2,4-D, as well as the ammonium sulfamate and sodium chlorate were each dissolved or dispersed directly into water with no added spreader or sticker. The concentrations were calculated on the basis of weight for each material.

Estimates of poison ivy cover were made visually before each treatment and in July of the first year following each application. They represent the percentage of the area of the plot covered with poison ivy foliage.

Table 1 gives the results obtained when 1 gallon lots of different concentrations of these materials were applied on June 19, 1946, to plots 100 sq. ft. in area containing approximately an 85% stand of poison ivy.

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TABLE 1.—THE EFFECTIVENESS OF HERBICIDAL SPRAYS APPLIED ON JUNE 19, 1946, TO AN 85% STAND OF POISON IVY

Herbicides	Percentage ground cover in July of the first year after application			
	Concentration of spray solution			
	500 ppm	1000 ppm	2000 ppm	100,000 ppm (10%)
	%	%	%	%
2,4-D methyl ester	—	2	2	—
2,4-D acid in carbowax	60	30	20	—
2,4-D ammonium salt	—	40	25	—
2,4-D triethanolamine salt	75	65	50	—
Ammonium sulfamate	—	—	—	2
Sodium chlorate	—	—	—	10

TABLE 2.—THE EFFECTIVENESS OF 1000 PPM. 2,4-D FORMULATIONS AND 10% AMMONIUM SULFAMATE WHEN APPLIED AS FOLIAGE SPRAYS TO AN 85% STAND OF POISON IVY, AT FOUR DIFFERENT DATES IN 1946

Date of treatment	Percentage ground cover in July of the first year after application			
	2,4-D formulation			Ammonium sulfamate
	Acid in carbowax	Triethanolamine salt	Methyl ester*	
	%	%	%	%
June 13	18	20	2*	2
July 16	80	80	10*	8
August 14	80	75	—	40
September 12	70	75	—	75

* The dates of treatment for the methyl ester were June 19 and July 31, respectively.

Table 2 gives the results obtained when one gallon of a 1000 ppm. solution of three 2,4-D formulations and one gallon of 10% ammonium sulfamate were applied in each of the months of June, July, August, and September, 1946, to plots 100 sq. ft. in area containing approximately an 85% stand of poison ivy.

There was considerable variation in the effect of different 2,4-D formulations on poison ivy. The methyl ester gave the best control with 2,4-D acid, 2,4-D ammonium salt, and 2,4-D triethanolamine salt following in that order. The control obtained from these limited tests with the methyl ester was equal to that from ammonium sulfamate and better than that from sodium chlorate. Increasing the concentration of the 2,4-D formulations increased the control of poison ivy but not on a proportional basis.

Corroborating the results from the 1945 trials, a better control was obtained from the June applications than from those made in July, August, or September. However, the results obtained from June applications of 2,4-D formulations were not so consistent as with those from ammonium sulfamate as large differences in control were obtained from treatments of 2,4-D made on June 13 and June 19, 1946, respectively.

The control of poison ivy obtained from these 1946 treatments with 2,4-D formulations was inferior to that from similar applications made on approximately the same dates in 1945.

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AN OCCURRENCE OF SOFT ROT IN PEPPERS AND ITS RELATION TO THE CORN BORER¹

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In late August and early September of 1947 in southern Ontario, an appreciable number of green, partially ripe and ripe pepper fruits of both sweet and hot varieties developed soft rot. Often as many as four or five pods on a single plant were affected. The disease was found in every planting in the Harrow district. At the time the disease was most abundant, the weather was hot and very humid—conditions that are conducive to soft rot development.

The first symptom observed was a softening and a water-soaking of the tissues of the pod at the stem end. The rotting progressed rapidly, usually down one side first, as seen in Figure 1. The interior of the fruit soon broke down to the consistency of a slimy fluid. Eventually the tough epidermis of the pod broke and released the contents over the parts of the plant underneath and on to the soil. Subsequently, the epidermis of the fruit dried into a parchment-like membrane and remained hanging over the end of the peduncle. In most cases the soft rot advanced down the peduncle and caused a rotting of its cortical tissues. Occasionally the main stem was attacked at the point of origin of the fruit stalk. Branches that arose from this region often split at this point later in the season.

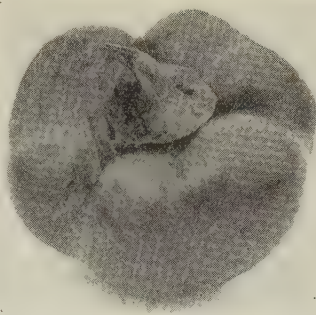
Erwinia carotovora Jones was isolated from fruits that showed incipient stages of soft rot as well as from fruits in which the rot had progressed further. The organism was also isolated from fruit stalks that showed tissue decay. The disease was readily reproduced by inoculating green and ripe pepper fruits through scalpel wounds. Infection was not obtained by inoculating non-wounded peppers.

During an inspection of a field in August, it was brought to the attention of the writer that insects were often found in some of the peppers. Specimens were collected and the insect was identified by the Dominion Entomological Laboratory, Chatham, Ontario, as the larval stage of the European corn borer (*Pyrausta nubilalis* Hübner). In September, many fruits that showed incipient infections were examined. In every case a small insect burrow was found in the wall of the fruit, usually at the stem end. When the pod was cut open, a corn borer larva was discovered inside but, when the pepper showed extensive decay, the insect was not found in the fruit. Frequently, however, a larva was found inside another pod on the same plant.

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FIGURE 1. Fruit of sweet pepper, showing soft rot lesion beginning at stem end and involving one side. Note larval burrow and castings on the peduncle.



The pathogen *E. carotovora*, being a wound parasite, is incapable of attacking healthy, uninjured tissue. It seems evident from the observations that the corn borer larva provided an infection court in the pepper fruit for the soft rot pathogen. Johnson (1) found that cabbage maggot larvae that had been feeding upon decaying cabbage tissue, when transferred to fresh cabbage, inoculated the leaves with soft rot bacteria and reduced them to a rotten mass. Similarly, it would be expected that corn borer larvae become contaminated with bacteria when they leave a partially-rotted pepper fruit. Such larvae, as they burrow into another fruit, would inoculate the succulent tissue and would thereby become important disseminating agents of the pathogen in addition to providing an infection court. This hypothesis would seem to explain the occurrence of several rotted fruits on a single plant and the disease-free condition of adjacent plants.

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BOOK REVIEW

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This catalogue, covering the results of biological testing of some 10,000 materials, forms Volumes 7 and 8 of the "*Annales Cryptogamici et Phytopathologici*" edited by Dr. Frans Verdoorn, Managing Editor of "*Chronica Botanica*".

The present volume, the first of two, deals with those materials tested for the control of insects. The compilation is the result of a search of many journals, textbooks, patents, as well as heretofore unpublished information contributed by co-operating industrial and private testing laboratories. The major emphasis is placed on the less commonly used materials with no effort made to cover the literature on the widely used insecticides. The compounds are arranged according to a system of coding whereby each compound has a "code number" made up of the numbers assigned to each constituent group present in the compound. Although this system is fully explained in the introduction many will find difficulty in locating the desired materials. However, a complete alphabetical index of all compounds, by name, is to be given at the end of Volume II.

The following information is provided for each material: name, formula, synonyms, insects against which the compound has been tested, results of toxicity tests, and literature citations. The 1,513 literature references are arranged in numerical order and alphabetically by authors. An index, listing the patents by countries and by numerical order, is also given.

This catalogue, by bringing together the published literature on materials tested, and by giving a brief summary of the results achieved, will be of real value to all research workers dealing with insecticides.

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